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NOVEMBER, 1922

Resumen por el autor, E. R. Clark.

Reacciones de los capilares linfáticos aislados experimentalmente en la cola de las larvas de anfibios.

El autor ha separado porciones de los capilares del resto del sistema linfático, observando sus reacciones en el renacuajo vivo. Las porciones así separadas mantuvieron su vitalidad e identidad como tales linfáticos y eventualmente se reunieron con el sistema linfático. El tiempo transcurrido entre la separación y reunión ulterior con dicho sistema varía entre 14 y 21 dias. Mientras están separados, los capilares aislados envían procesos y los retiran, absorbiendo y eliminando líquido, como demuestran los cambios en su calibre. No existe tendencia alguna a transformarse en otros tipos de células. Estos resultados prestan apoyo a la idea que supone al endotelio linfático como un tejido especifico. Los vasos sanguíneos cortados se reunieron al cabo de tres dias de separación. Los nervios cortados volvieron a reunirse al cabo de cinco dias y las porciones separadas, que presentaban aspecto granular, bien proto adquirieron su aspecto normal.

Translation by José F. Nonídez
Cornell Medical College, New York.

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
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REACTIONS OF EXPERIMENTALLY ISOLATED LYMPHATIC CAPILLARIES IN THE TAILS OF AMPHIBIAN LARVAE

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TEN FIGURES

The normal growth of blood vessels and of lymphatics has been thoroughly studied by observation of the transparent tails of living amphibian larvae and the results reported elsewhere (E. R. Clark, '09, '12). By watching individual cells and vessels consecutively for days and even weeks, the method of growth of blood vessel and lymphatic capillaries, after their primary differentiation by sprouting, i.e., by extension from previously formed endothelium, has been firmly established. The manner in which the pattern of blood capillaries changes with changes in the circulation and the development of certain vessels of an indifferent capillary plexus into venules and arterioles are also problems which have been studied by direct observation of the living (E. R. Clark, '18).

In the course of these studies on blood vessels, an experiment was performed in which a blood capillary was completely isolated by cutting through its connections. Two days later this isolated capillary was found to have formed an anastomosis with one of the near-by circulating blood vessels, thus showing that a capillary which has become isolated does not lose its blood-vascular endothelial properties and may be reincorporated in the vascular system. This vessel was located near the margin of the fin in a region where active new formation of vessels was taking place.

This method of observation of a transparent region, such as the tadpole's tail, using chloretone anaesthesia to keep the animal motionless and an upright glass chamber such as has been

described (Clark, '12), seemed to afford a good opportunity for studying the regeneration of blood vessels and lymphatics, and also of nerves, after experimental injury.

DESCRIPTION OF EXPERIMENT

The following experiment was performed. Under the binocular dissecting microscope, a longitudinal cut was made in one of the tail fins of a Fowler's toad larva, anaesthetized with chloretone 1 to 3000. The cut was made with a sharp knife. Such a cut severed a number of blood capillaries, lymphatics, and nerves.

Immediately after the operation the tadpole was transferred to the observation chamber and the region studied under the compound microscope. It was found that the epidermal cells became opaque soon after the injury, drew together, and heaped up over the line of incision. Soon after making the cut, the processes of a number of the near-by connective-tissue cells became shorter and stiffer. Within fifteen minutes the leucocytes in the neighboring blood vessels began to adhere to the walls. Half an hour after the injury a distinct shrinkage of the corners of the cut was noticed. At this time also the leucocytes in the opposite fin near the region of the cut were observed to be sticking to the walls of the near-by blood vessels.

When a tadpole, operated on in this manner, was returned to fresh water and observed on the following day—approximately seventeen hours later—it was found that the cut surfaces had united, the epidermal layers having apparently healed by first intention, along the entire extent of the cut. A number of leucocytes had collected near the line of healing. Figure 1 is a low-power drawing of such a region on the day following a cut of this nature.

THE BLOOD VESSELS

When the region of a simple cut through the tail fin was observed on the day following the operation, a number of the blood vessels were found to end blindly near the line of healing. Observations showed a very slow circulation in the blood vessels

on the marginal side of the cut—the blood flowing for part of the time in one direction and then in the other. It should be noted that in this type of operation there were no entirely isolated capillaries as in the experiment reported above (Clark, '18), but instead a portion of a blood-vessel plexus was separated from another part with which it had been formerly connected (fig. 1).

On the following day the area of circulation had increased somewhat in the part of the plexus distal to the cut and the current flowed in a caudal direction.

On the next day—three days after making the incision—the cut blood vessels had all united with each other and the plexus was continuous, just as before the operation, and the circulation had been reestablished.

It is clear, then, from this experiment, just as was the case with the operation which isolated a single blood capillary, that when two parts of a blood-capillary plexus in amphibian larvae are separated from each other, the vessels reunite and the circulation is reestablished within the space of two or three days. The healing of such blood vessels, cut through in this manner, is accomplished by the same process of sprouting of the endothelium which characterized the normal process of growth in the tadpole's tail.

THE LYMPHATIC CAPILLARIES

The lymphatics cut through in this experiment were followed carefully and records made of them on successive days. Three days after the operation, when the severed blood vessels had all reunited, the cut lymphatics were still isolated. The proximal ends of these cut-off lymphatics were somewhat widened, while the remainder of the vessels were either normal in size or smaller than normal. During the succeeding days this widening of the proximal end of such an isolated lymphatic was noticed to be present at times, while at others the lumen was found to have returned to its normal caliber and, still later, the widening was again observed, thus showing that the fluid passes in both directions through the endothelium of such cut-off lymphatics. Aside from this change in the size of the lumen, no abnormalities

in the appearance or contour of such a cut-off lymphatic were noticed during the period of isolation. Such an isolated vessel, however, retains the power of growth and may even extend in a distal direction as was the case in the vessel recorded in figures 2 and 3.

Tadpoles operated on in this manner were followed for more than two weeks after the experiment with daily observations. At ten days after the operation, isolated lymphatics were still present and their appearance did not differ from that found soon after making the cut. In one such experiment a week or more after the operation the near-by lymphatic capillary with which the isolated vessel had formerly been connected began to send out a new sprout in the direction of the cut-off lymphatic. Such a sprout was at first solid and resembled exactly the sprouts of new growing lymphatic capillaries. On the twelfth day after the original cut, a fine thread from one of these new lymphatic branches had reached the isolated vessel and had united with it (figs. 2, 3, and 4).

In another specimen a lymphatic vessel was severed accidentally by the injection of a comparatively large globule of paraffin oil. Figures 5 to 10 are a series of camera-lucida sketches of this region, beginning with the thirteenth day after the injection (August 9th), when the lymphatic vessel (*B*) was still isolated. The lymphatic with which the isolated portion had originally been connected is shown at the top of the drawing and labeled *A*, but another lymphatic (*C*) had grown nearer to the cut-off vessel during the interval. During the subsequent days the nearer lymphatic (*C*) extended in the manner shown in figures 6 and 7. At first, as recorded on August 12th, the new sprout was apparently growing in a different direction instead of toward the isolated lymphatic (fig. 6). On the following day, however, this sprout was withdrawn and another one had grown out which approached nearer to the isolated vessel, while the isolated vessel itself was observed to send out a new sprout toward the approaching lymphatic outgrowth (fig. 7). On August 14th —eighteen days after the original experiment—these new sprouts from the two lymphatics had united (fig. 8). The connection

was at first narrow and solid, but during the succeeding three days it gradually enlarged, and finally, on August 20th (fig. 10), a continuous lumen was observed, and thus, twenty-four days after the original experiment which isolated it, this lymphatic capillary became completely reincorporated in the lymphatic system.

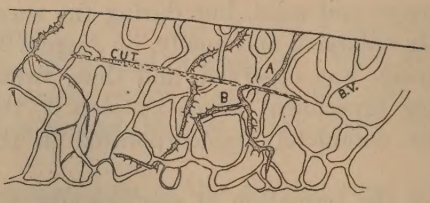
The picture of the formation of new sprouts, which were at first solid and later acquired a lumen, is identical with that found in the normal growth of lymphatic capillaries as described in earlier observations (Clark, '09, '12). It is evident from the present observations that lymphatic capillaries, experimentally isolated from the rest of the lymphatic system, retain their vitality and an independent power of growth for at least three weeks after the separation and are eventually reincorporated in the lymphatic system by a process which does not differ from the normal method of growth.

This cut through the tail also severed a number of peripheral nerves. The separated nerves became granular in appearance, with their finer processes invisible. On the fifth day following the experiment these nerves had reunited and recovered their normal appearance. However, further studies are necessary before a positive statement should be made as to the exact mode and extent of regeneration in these severed peripheral nerves of amphibian larvae.

DISCUSSION AND CONCLUSION

It has long been known that the severed capillaries of a blood-vessel plexus will regenerate and reunite across the gap made by an incision. A histological picture of the changes which occur in the case of healing of the blood vessels of the intestine in adult mammals has been given recently by Sabin ('20). She described the blood-vessel endothelium in such cases as resuming its embryological character and finds that solid angioblasts are formed from which blood vessels develop by a process of liquefaction of the protoplasm (cf. Halsted, '21).

The present study, made in the living transparent specimen, shows that when one portion of a circulating blood-vessel plexus



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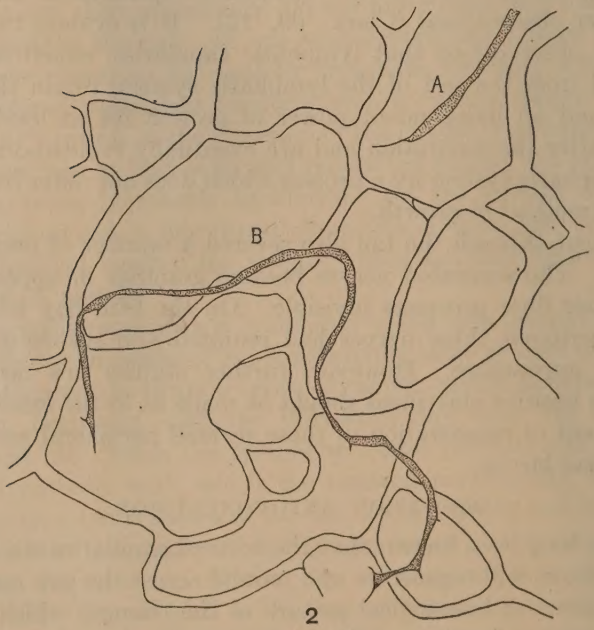

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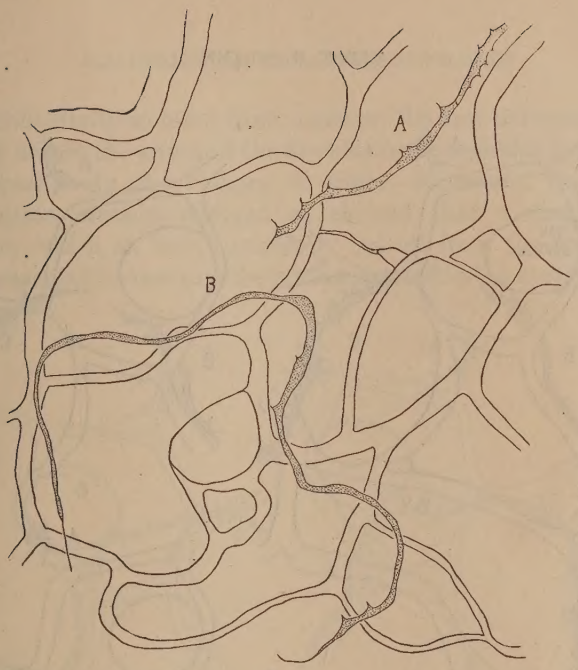
Fig. 1 Low-power sketch of the region of a cut through the dorsal fin of a tadpole, drawn eighteen hours after making the incision. The epidermal layers have healed. A number of blood vessels and lymphatics, cut across, end blindly at the line of healing. The lymphatics are dotted in this and succeeding figures. *B.V.*, blood vessel; *Lym.*, lymphatic; *A*, lymphatic vessel; *B*, isolated portion of lymphatic *A*.

Fig. 2 A small region from the same specimen shown in figure 1, drawn August 8th (five days after the original cut). The lymphatics shown here are those which were labelled *A* and *B* in figure 1. The proximal projection, present in the isolated lymphatic *B* on the day after the cut, has been withdrawn. The severed blood vessels have all healed and new blood capillaries have formed in the region.



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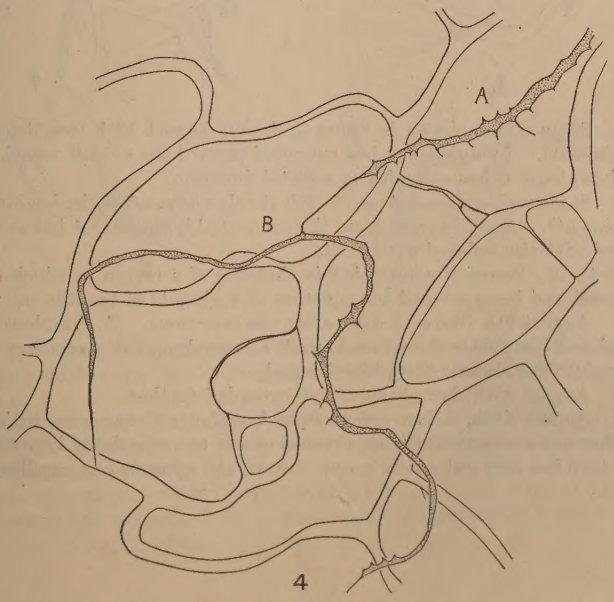

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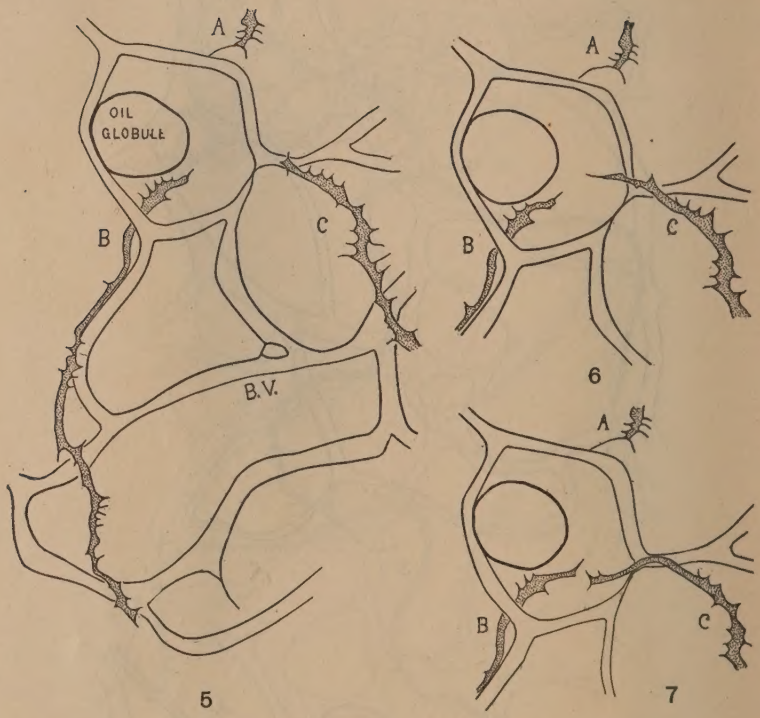

Fig. 3 Same region shown in figure 2, drawn August 13th (ten days after making the cut). Lymphatic *A* has extended toward the cut-off vessel. The isolated lymphatic *B* has extended in a distal direction.

Fig. 4 Same region drawn August 15th (twelve days after the beginning of the experiment). A solid sprout from the connected lymphatic *A* has extended and united with the isolated portion *B*.

Figs. 5 to 10 Series of camera-lucida sketches of a region in which a lymphatic vessel had been severed by injection of a globule of paraffin oil.

Fig. 5 August 9th (thirteen days after the injection). *A*, lymphatic from which isolated lymphatic *B* was separated; *C*, new lymphatic capillary which has grown into the region; *B.V.*, blood vessel.

Fig. 6 August 12th, lymphatic *C* has extended further.

Fig. 7 August 13th. New sprout from lymphatic *C* has been withdrawn and another one sent out which approaches nearer to the isolated lymphatic *B*, which in turn has sent out a new sprout toward the approaching capillary.

is experimentally isolated from another, the two portions reunite rapidly across the gap and the circulation is reestablished within a comparatively short space of time. Moreover, the former observation already referred to showed that a single blood capillary which as been completely cut off from the rest of the blood vascular system may be reincorporated in the system within

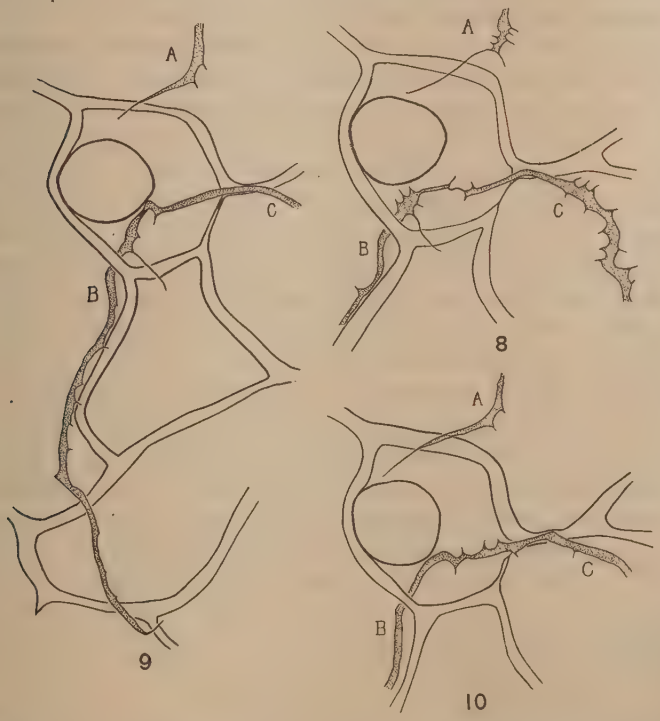

Fig. 8 August 14th (eighteen days after the separation of the lymphatic *B*). Shows a solid connection uniting the severed lymphatic *B* with the new sprout from *C*.

Fig. 9 August 16th. The connection between *B* and *C* is larger, but is still solid. The distal portion of the formerly isolated lymphatic is narrower than before (compare with fig. 5).

Fig. 10 August 20th (twenty-four days after the original experiment). A lumen is now present at the point of union of lymphatics *B* and *C*.

twenty-four hours after the experiment which isolated it. The method by which the blood capillaries reunite is identical with the process of normal growth of blood vessels, namely, by the sending out of solid protoplasmic sprouts from the blood vascular endothelium.

The exact mode of regeneration of lymphatic vessels has never been definitely established. Halstead ('21) in his recent article on elephantiasis, shows that the blood vessels have reunited across a gap within a few days and assumes that the lymphatics have also anastomosed in this space of time, but no injections were made to test this point. On the other hand, A. W. Meyer ('06) concludes from his investigations that lymphatics have no power of regeneration. Meyer ligated and cut the two largest lymphatic trunks anterior to the saphenous vein in four adult dogs and, in four other animals, he resected from 3 to 5 mm. of the trunk. The specimens were killed and examined fourteen to ninety-one days after the operation. No evidence of the regeneration of these lymph ducts was found, either by injection or by microscopic examination.

From the present experiment it is clear that lymphatic capillaries of amphibian larvae which are isolated experimentally from their connection retain their vitality and their power of growth for comparatively long periods of time (three weeks and more) and are eventually reincorporated in the lymphatic system. During their period of isolation there is evidence of the passage of fluid in both directions through the endothelial wall. The regeneration, or better, the healing of such severed lymphatic capillaries is very much slower than in the case of blood-vessel capillaries separated in a similar manner.

These studies bring new evidence in favor of the specificity of blood-vessel and of lymphatic endothelium, for they show that both of these types of endothelium remain specific even when completely isolated from the remainder of their respective systems. It was shown in a previous paper that an isolated blood capillary remains specific and reunites with the blood vascular system. In this study it is found that lymphatic capillaries when isolated retain their specific modes of reaction and reunite

with the lymphatic system. There is no indication of the transformation, at least at this stage and in this animal, of blood-vessel into lymphatic endothelium or vice versa, nor of either type of endothelium into indifferent mesenchyme cells or wandering cells.

The suggestion made by F. T. Lewis ('06) that, in very early stages, blood vessels may become cut off and transformed into lymphatic endothelium—an hypothesis which has been neither proved nor disproved—still remains a possibility for earlier stages. However, it seems to the author that this study makes that possibility a little less probable.

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Resumen por le autor, A. Kuntz.

Sobre la existencia de arcos reflejos en los plexos mientérico y submucoso.

El presente estudio se basa primariamente sobre preparaciones del estómago e intestino del gato, teñidas con el azul de metileno. Las fibras preganglionares viscerales eferentes y las que se originan en el plexo submucoso establecen sinapsis con las neuronas del plexo mientérico. Las sinapsis de dos neuronas en el mismo ganglio y las de dos neuronas en ganglios separados del mismo plexo existen también en el plexo mientérico y en el submucoso. Las sinapsis de neuronas de los plexos entéricos indican la existencia de arcos reflejos locales. El presente estudio no suministra pruebas de la existencia de redes nerviosas en las paredes del tubo digestivo. Las fibras que se originan primariamente en el plexo submucoso terminan en el epitelio gástrico e intestinal. Estas fibras son probablemente las dendritas de neuronas del plexo submucoso. Los hallazgos anatómicos mencionados suministran una base estructural para la interpretación racional de todas las actividades usuales del estómago y del intestino, con la excepción de la contracción rítmica. El autor llama la atención hacia los trabajos de Magnus, Gunn y Underhill, y Alvarez y Mahoney, los cuales indican la naturaleza miogénica de estas contracciones.

Translation by José F. Nonidez
Cornell Medical College, New York

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ON THE OCCURRENCE OF REFLEX ARCS IN THE MYENTERIC AND SUBMUCOUS PLEXUSES

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NINE FIGURES

INTRODUCTION

The myenteric and submucous plexuses constitute one of the most obscure portions of the sympathetic nervous system. Much has been written about them, but relatively few anatomical investigations have dealt with them intensively. The morphological characters of the ganglia and fibrous commissures, including the histological structure of the neurons, composing these plexuses have been described in detail. However, the available anatomical data give us little exact knowledge regarding the interrelationships of the neurons or of the relationships of the latter to the general visceral efferent and afferent neuron systems. The present status of our knowledge regarding the enteric plexuses is well characterized by Ranson ('21) when he says, "since next to nothing is known about the structure of these plexuses and the interrelations of the nervous elements which compose them, the problems relating to the control of the movements of the alimentary tract are very baffling."

Ranson ('21) has recently reviewed the literature bearing on the functional relationships of the enteric plexuses, including references to the more important anatomical studies. Erick Müller ('21) has also reviewed the literature bearing on the microscopic structure of these plexuses. Therefore, a systematic review of the literature will not be undertaken in this paper.

The myenteric plexus is composed of numerous somewhat irregular, flattened or lens-shaped ganglia interposed between the longitudinal and circular muscle layers and connected with

each other by commissures of non-medullated fibers. The submucous plexus comprises numerous small ganglia located in the submucous layer and, likewise, connected with each other by commissures of non-medullated fibers. Similar commissures also connect the myenteric and submucous plexuses with each other. Non-medullated fibers may also be traced from the ganglia in the myenteric plexus into the longitudinal and circular muscle-layers where they terminate on muscle fibers. Likewise, non-medullated fibers may be traced from the ganglia of the submucous plexus into the muscularis mucosae, among the gastric and intestinal glands, and into proximity with the gastric and intestinal epithelium.

These general morphological characters of the myenteric and submucous plexuses have been described repeatedly. Therefore, a detailed description would be superfluous at this time. It is the writer's purpose in the present paper to set forth anatomical evidence of the occurrence of local reflex arcs within the walls of the stomach and intestine and to point out, as far as possible, the anatomical basis for a rational interpretation of the physiological activities of these organs.

METHODS

The observations set forth in the present paper were made almost exclusively on methylene-blue preparations of the stomach and small intestine of the cat. The writer has during the past ten years repeatedly attempted, by the use of both intravitam and impregnation methods, to secure good histological preparations of the enteric plexuses, but with only indifferent success. The preparations most servicable for the study of the morphology of the neurons were secured by the use of the pyridine-silver method. Those most servicable for the study of synapses within the enteric plexuses and fiber terminations on muscle, gland, and epithelial cells were secured by the intravitam methylene-blue method.

MICROSCOPIC STRUCTURE

Morphology of neurons

Classifications of the neurons in the enteric plexuses, as well as in other portions of the sympathetic nervous system, according to morphological types have been attempted repeatedly. Dogiel ('95–'99) described neurons of two distinct types in these plexuses. Those of type I were characterized by numerous short, frequently branching dendrites and an axon which could be traced into the musculature. Those of type II were characterized by few long, infrequently branching dendrites and an axon similar in appearance to the dendrites. Dogiel interpreted the neurons of type I as motor and those of type II as sensory in character. As early as 1889 Cajal discovered, in addition to the larger neurons in the ganglia in the walls of the digestive tube, certain small spindle-shaped and angular cells with varicose processes which occur in all the layers and are not limited to the ganglia. He designated these elements as interstitial cells, but interpreted them as free neurons. La Villa ('97, '98) studied these elements in methylene-blue preparations and also interpreted them as neurons. In his later work Cajal ('09) still maintained that the interstitial cells are nervous elements and emphasized the presence in them of neurofibrillae, as shown in material prepared by silver-impregnation methods. Dogiel ('95) interpreted these interstitial cells not as neurons, but as connective-tissue elements. Both Heidenhain ('11) and Huber ('13) concur in this interpretation. L. R. Müller ('11) could differentiate two types of neurons in the myenteric, but not in the submucous plexus. In the former plexus the neurons of the one type lie free in small lacunae and give rise to numerous processes. Those of the other type lie close to the musculature and give off small dendrites to it. He suggests that the neurons of the former type may be sensory and those of the latter type motor. However, he clearly points out that there are no data available which warrant a positive conclusion regarding functional differences in these neurons. Erick Müller ('21) claims to have substantiated the conclusion of Dogiel that the neurons in the myenteric and sub-

mucous plexuses conform to two morphological types. Furthermore, he identifies the interstitial cells of Cajal with the neurons of type II of Dogiel. However, he does not accept Dogiel's interpretation of the functional relationships of the neurons of this type.

The present study contributes little to our knowledge of the general morphology of the neurons in the enteric plexuses. There is a wide range of variation in the size, form, and other characters of the neurons in both plexuses. The average size of the neurons in the myenteric plexus is somewhat greater than the average size of those in the submucous plexus. Both neurons with short, frequently branching dendrites and neurons with longer, less frequently branching dendrites occur in the myenteric plexus. Probably neurons of both types occur also in the submucous plexus. In the latter plexus, neurons with relatively long dendrites clearly preponderate. However, many neurons in both plexuses conform closely neither to the one nor to the other of these types. The present study does not warrant the conclusion that the neurons in these plexuses can be classified according to two distinct morphological types. Regarding the interstitial cells, the writer must agree with those authors who have interpreted them as connective-tissue elements. These elements are brought out more clearly in the pyridine-silver than in the methylene-blue preparations used in this study. They are especially abundant in the submucous layer. They differ so widely, both in their staining reactions and in their structure, from the neurons in the ganglia that the conclusion that they are nervous elements seems unwarranted.

Synaptic relationships

The problems regarding the interrelationships of the neurons in the enteric plexuses and their relationships to the general visceral efferent neuron system are of primary importance. Available anatomical data throw little light on them. Synapses within the walls of the digestive tube have rarely been observed. The writer described such synapses in an earlier paper ('13).

However, the fibers whose terminations in relation to neurons were observed could not be traced from their origin; consequently, they could all be interpreted as preganglionic fibers. Some of the methylene-blue preparations used in the present study are especially favorable for the study of synapses by reason of the unequal reaction of the neurons to the stain. In these preparations some of the neurons are stained intensely, while others are stained very lightly or not at all. In many of the ganglia but few of the neurons present are apparent. The terminal portions of the axons commonly react intensely to the stain; consequently, synapses may be observed most readily on neurons which are stained very lightly.

The synapses observed in this study are all similar in character. As the axon approaches the neuron in relation to which it terminates, it divides into several branches which embrace the cell body. These branches give rise to smaller branches with which they form a more or less complex pericellular arborization. In many instances the source of the axon involved in the synapse cannot be determined. In others the source of the axon may be inferred from the position and relationships of the commissure in which it approaches the ganglion where the synapse occurs. In still others the synaptic relationship involves two neurons located in the same ganglion.

The synaptic relationships indicated above are illustrated in the accompanying figures. Figure 1 is a camera-lucida drawing illustrating several synapses in a small ganglion in the myenteric plexus in the small intestine. The fibers which are shown entering this ganglion are contained in a commissure which connects it with a small ganglion in the submucous plexus. Doubtless, they arise in the latter ganglion. Axons *a* and *b* either represent preganglionic visceral efferent fibers or axons which arise in another ganglion in the myenteric plexus. Figure 2, likewise, is taken from a longitudinal section of the small intestine. It illustrates a similar synapse involving an axon which probably arises in the submucous plexus and a neuron in the myenteric plexus. Synapses which clearly involve axons which arise in the myenteric plexus and neurons in the submucous plexus were

not observed. However, synapses occur also in the submucous plexus. Figure 3 is a camera-lucida drawing illustrating two synapses in approximately exact spatial relation to each other and to the muscularis mucosae in the stomach. Inasmuch as the axons involved in these synapses run nearly parallel with the muscularis mucosae as far as they may be traced and approach the respective neurons in opposite directions, both cannot be interpreted as preganglionic visceral efferent fibers. Probably both synapses involve only neurons in the submucous plexus. Synapses involving two neurons located in the same ganglion in the myenteric plexus are illustrated by camera-lucida drawings in figures 4 and 5. Synapses involving two neurons located in the same ganglion in the submucous plexus are illustrated in the same manner in figures 6 and 7. In all these cases the axon may be traced without interruption from its origin to its termination. The neuron from which the axon arises is stained intensely; the one in relation to which it terminates is stained very lightly. The majority of the neurons in the ganglia are stained very lightly or not at all. These conditions render the preparations

Fig. 1 Camera-lucida drawing illustrating synapses in a ganglion in the myenteric plexus in the small intestine of the cat, longitudinal section. Peripheral line indicates the periphery of the ganglion. *a* and *b*, probably preganglionic visceral efferent fibers; *c*, fibers arising in the submucous plexus.

Fig. 2 Camera-lucida drawing illustrating synapse of a fiber arising in the submucous plexus with a neuron in a small ganglion in the myenteric plexus in the small intestine of the cat, longitudinal section. Peripheral line indicates the periphery of the ganglion and its relation to a commissure connecting it with the submucous plexus.

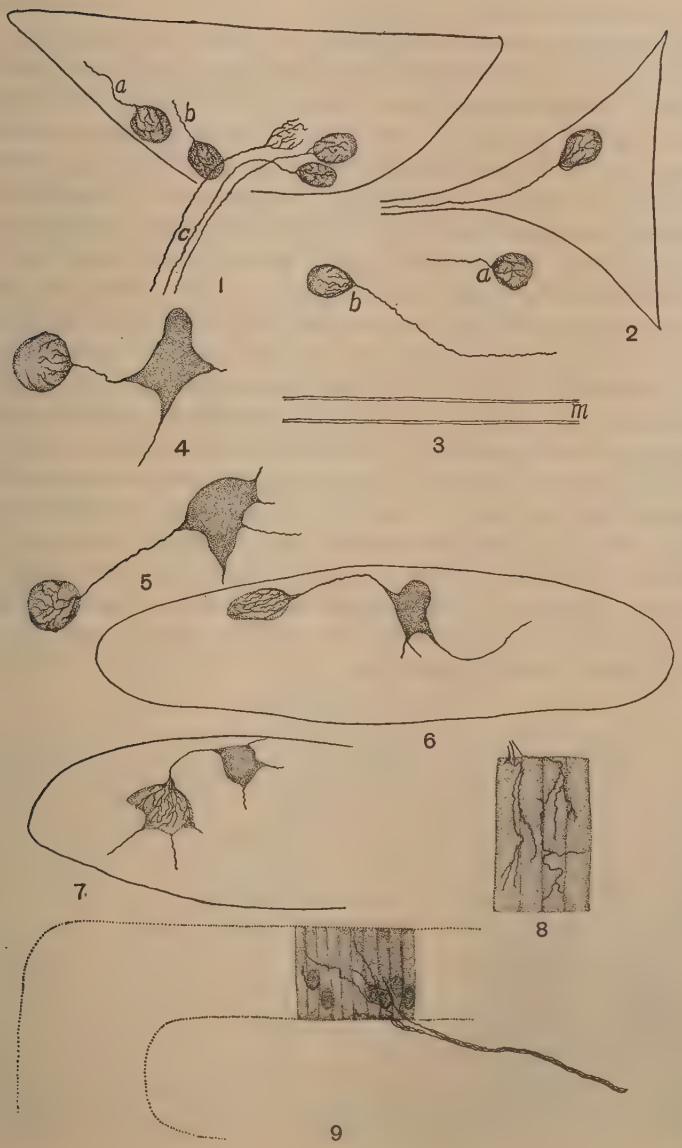
Fig. 3 Camera-lucida drawing illustrating synapses (*a* and *b*) in the submucous plexus in the stomach of the cat in approximately exact spatial relation to each other and to the muscularis mucosae (*m*).

Figs. 4 and 5 Camera-lucida drawings illustrating synapses involving two neurons in the same ganglion in the myenteric plexus in the small intestine of the cat.

Figs. 6 and 7 Camera-lucida drawings illustrating synapses involving two neurons in the same ganglion in the submucous plexus in the small intestine of the cat. Peripheral lines indicate the peripheries of the ganglia in longitudinal section.

Fig. 8 Camera-lucida drawing illustrating terminations of sympathetic fibers in the intestinal epithelium at the distal end of a villus.

Fig. 9 Camera-lucida drawing illustrating terminations of sympathetic fibers in the intestinal epithelium near the distal end of a villus.


a
b
c
1
b
a
2
4
m
3
5
6
7
8
9

very favorable both for the observation of the uninterrupted axon and its terminal arborization.

The synaptic relationship described above, which involves an axon in a commissure which connects the submucous with the myenteric plexus and a neuron located in a ganglion in the latter plexus, strongly suggests a local reflex arc of which the afferent element is a neuron in the submucous plexus. Obviously, two neurons which are located in the same ganglion and sustain a synaptic relationship to each other cannot be interpreted as the terminal elements in a visceral efferent chain. They can only be interpreted as constituting a partial or complete local reflex arc. Possibly such reflex arcs involve a third neuron. The data at hand afford no positive evidence on this point. The conditions stated above which make possible the observation of synapses would practically preclude the observation of two successive synapses in an arc composed of three neurons, even though such arcs were known to be present. Whether these local reflex arcs comprise two or three neurons is a matter of minor importance. The synaptic relationships involving two neurons, as described above, demonstrate a nervous mechanism within the walls of the stomach and intestine through which local reflexes may be carried out.

Fiber-terminations

Terminations of sympathetic fibers on muscle and gland cells in the walls of the digestive tube have been described repeatedly. Their occurrence is generally admitted. Terminations of sympathetic fibers in the gastric and intestinal epithelium have also been observed. Such fiber-terminations were described by Sacussew ('97) in the intestinal epithelium of the fish and by R. Müller ('08) in the gastric epithelium of the frog. The writer ('13) also described fiber-terminations on the gastric and intestinal epithelium of the cat. More recently, Erick Müller ('20, '21) described fiber-terminations in the digestive epithelium in Selachii and in the chick. Nevertheless, the present attitude of the majority of anatomists regarding the occurrence of terminations of sympathetic fibers in the gastric and intestinal epithelium seems to be one of scepticism.

The preparations used in the present study admit of no doubt regarding the occurrence of sympathetic fiber-terminations in the gastric and intestinal epithelium in the cat. Non-medullated fibers penetrate the muscularis mucosae and descend into the gastric folds and plicae and the intestinal villi in large numbers. Many of these fibers ramify among the glands in the mucosa, others supply the intravillus muscle fibers. However, many of them approach the epithelium and give rise to terminal branches which ramify among the epithelial cells. Camera-lucida drawings of such fibers in relation to the epithelium are presented in figures 8 and 9. Fiber-terminations in the gastric and intestinal epithelium were described by the writer in essentially the same manner in the earlier paper referred to above. However, it is now clear that the terminal branches of such fibers ramify more deeply among the epithelial cells and approach the free ends of the latter more closely than they could be traced in the earlier study. Obviously, it is impossible in sections to trace individual fibers from their cells of origin in the enteric plexuses to the gastric or intestinal epithelium. However, individual fiber bundles may be traced from the submucous plexus into proximity with the epithelium where certain of the fibers give rise to terminal branches which ramify among the epithelial cells. In view of the current teaching, it might be argued that the fibers which terminate in the gastric and intestinal epithelium are not sympathetic, but general visceral afferent fibers. Clearly, their identity with the latter cannot be demonstrated. Furthermore, they are present in such abundance that they could not be accounted for as visceral afferent fibers contained in the vagus and spinal nerves, even though we should admit of the most profuse branching. Neither can they be differentiated histologically from the other fibers which penetrate the muscularis mucosae. The majority of the fiber bundles involved also arise in the submucous layer. Doubtless, the fibers which ramify among the gland cells represent axons. Those which approach the epithelium present the same histological appearance; however, inasmuch as they terminate in the epithelium, they are, doubtless, afferent in character and probably represent dendrites.

DISCUSSION

According to current anatomical teaching, the sympathetic nervous system is essentially an efferent system. The visceral efferent chain comprises a preganglionic neuron located in the central nervous system and a postganglionic neuron located in a sympathetic ganglion. Afferent visceral impulses which are mediated by the general and special visceral afferent neurons may be transmitted to the preganglionic visceral efferent neurons in the cranial and spinal medulla and result in visceral reflexes. The occurrence of visceral reflex arcs which comprise only sympathetic neurons is not generally admitted.

There are neither anatomical nor physiological data available which either suggest or demand the presence of afferent sympathetic neurons in any part of the sympathetic nervous system except the plexuses in the walls of the hollow viscera. Furthermore, exhaustive anatomical studies of other parts of the sympathetic system have failed to reveal synaptic relationships except those of preganglionic visceral efferent fibers to sympathetic neurons. On the other hand, there is a large volume of physiological data available regarding the activities of the digestive tube which cannot be intelligently interpreted unless we admit the occurrence of afferent sympathetic neurons in the enteric plexuses. Consequently, some physiologists have disregarded the current anatomical teaching and have assumed the presence of local reflex arcs within the walls of the digestive tube.

As stated above, Dogiel described sympathetic neurons of two distinct morphological types which he interpreted, respectively, as motor and sensory in character. He believed that neurons of both types occur in all parts of the sympathetic nervous system. As pointed out above, there are no facts available which indicate the occurrence of sensory (afferent) sympathetic neurons in any part of the sympathetic system except the plexuses in the walls of the hollow viscera. Neither have the later attempts of Cajal ('05, '06), Michailow ('08, '11), and others to classify sympathetic neurons according to morphological types resulted in any notable advances in our knowledge regarding their functions and interrelationships. The fact that certain investigators,

especially Michailow, have described sympathetic neurons of more than two morphological types only emphasizes the diversity of these elements and indicates that there are many sympathetic neurons which conform closely neither to type I nor to type II of Dogiel. Therefore, the correlation of motor and sensory functions with distinct morphological characters of sympathetic neurons seems unwarranted.

There has long been a tendency on the part of certain investigators to regard the enteric nervous mechanism as composed, at least in part, of nerve nets characterized by actual continuity of protoplasm between the constituent cellular elements. Bethe ('03) and R. Müller ('08) described the enteric plexuses in the frog as consisting of true nerve nets. These authors probably did not regard these plexuses as differing fundamentally from other parts of the sympathetic nervous system. They were still unconvinced of the validity of the neuron theory. Erick Müller ('20) described the enteric plexuses in the Selachii as composed exclusively of nerve nets. In a later paper ('21), he described these plexuses in birds and mammals as consisting in part of nerve nets and in part of free neurons. Obviously, this author regards the enteric nervous mechanism as differing fundamentally from the other parts of the sympathetic nervous system. He says the cells in the enteric plexuses are cells sui generis ("Die Elemente der Darmgeflechte sind Zellen sui generis"). He emphasizes the presence in the enteric plexuses of neurons of both type I and type II of Dogiel, but does not agree with Dogiel that neurons of type II occur in the ganglia of sympathetic trunks and the prevertebral plexuses. Furthermore, he identifies the interstitial cells of Cajal, which, as pointed out above, were regarded by Dogiel and Huber as connective-tissue elements, with the neurons of type II of Dogiel.

Müller's conception of the morphology and functional relationships of the enteric plexuses is further conditioned by his conception of the development of these plexuses. He maintains that the neurons of type I are of vagus origin, while those of type II are of sympathetic origin, i.e., the cells which give rise to neurons of type I are displaced into the walls of the digestive

tube along the paths of the vagi, while those which give rise to neurons of type II are displaced from the sources of the cells which give rise to the ganglia of the sympathetic trunks. Consequently, he assumes that the neurons of type I remain functionally associated with the vagi and those of type II with the sympathetic nerves; i.e., the former subserve motor, the latter inhibitory functions. Both myenteric and submucous plexuses, according to Müller, comprise both free neurons and nerve nets. He further states that the myenteric plexus comprises only vagus elements in the stomach and both vagus and sympathetic elements in the intestine, while the submucous plexus, throughout the stomach and intestine, comprises only sympathetic elements. Müller assumes that conduction in the walls of the digestive tube is accomplished by the nerve nets, while local reflexes must be interpreted as axon reflexes.

As pointed out above, there is no secure basis for the classification of neurons in the enteric plexuses according to two distinct morphological types. Even though Müller's classification could be accepted, his contention that the neurons of type I are of vagus and those of type II of sympathetic origin could not be established. As shown by the writer in a series of earlier papers, the enteric plexuses arise primarily from cells which advance peripherally along the vagus nerves. This finding has been corroborated by Abel ('12) and Stewart ('20) and has recently been verified experimentally by the writer ('22). Müller admits that his findings regarding a contribution of cells of sympathetic origin are not so trustworthy as might be desired when he says, "das Verhältnis des sympathischen Anteiles habe ich nicht so ganau untersuchen können" (p. 241). We do not maintain that no cells of sympathetic origin become incorporated in the enteric plexuses. However, there is no reason to assume that such cells would differ essentially either from the cells of vagus origin or from those which remain in the sympathetic trunks and the prevertebral plexuses. Furthermore, cells of vagus origin are present in the submucous plexus before pathways are established along which cells of sympathetic origin could be displaced into the walls of the digestive tube. Therefore it,

could not be maintained that the submucous plexus comprises only neurons of sympathetic origin. If a distinct contribution to the enteric plexuses of cells of sympathetic origin which differ essentially from the cells of vagus origin cannot be established, there is, of course, no basis for Müller's theory of the functional relationships of these plexuses.

The present study affords no evidence of the occurrence of true nerve nets in any part of the digestive tube. Obviously, negative findings cannot disprove the occurrence of nerve nets. However, the finding of numerous synapses, especially synapses which involve two neurons in the same ganglion, lends strong support to the theory that these plexuses comprise only free neurons.

Our knowledge regarding the effects of vagus and splanchnic stimulation and the normal gastric and intestinal reflexes involving these nerves is much more complete than our knowledge regarding the rôle of the enteric plexuses. However, it is generally conceded that the control of the movements of the stomach and intestine is exercised largely by the myenteric and submucous plexuses, although many of the movements whose essential features are determined by the local nervous mechanism may be modified and regulated through the extrinsic nerves.

The known reflexes of the alimentary canal have recently been summarized by Ranson ('21). Many of these reflexes involve extrinsic nerves, others are carried out by the intrinsic nervous mechanism. As indicated by experimental observations, of which a few are referred to below, many of the normal activities of the stomach and intestine involve the more or less independent functioning of the enteric plexuses. According to Cannon ('97, '11), the intermittent discharge of food from the pyloris is controlled by the local nervous mechanism. According to Carlson ('16), the essential features of the hunger contractions are determined by the local neuromuscular mechanism, although these contractions may be modified and regulated by extrinsic nerves. All the movements of the intestine persist after the extrinsic nerves are severed. According to Cannon ('12), the myenteric reflex is responsible for peristalsis in the small intestine,

but not for gastric peristalsis nor for antiperistalsis and segmentation in the intestine.

All the phenomena noted above and others which might be mentioned seem to require a local reflex mechanism within the walls of the stomach and intestine. On the other hand, the rhythmic contractions of the stomach and intestine persist following the administration of nicotin in doses sufficient to prevent conduction through synapses. Furthermore, conclusive anatomical evidence of the occurrence of synapses in the enteric plexuses, except those of preganglionic with postganglionic efferent neurons, was not forthcoming. Consequently, many physiologists as well as anatomists have not been willing to admit the occurrence of local reflex arcs in the enteric plexuses. Doubtless, these circumstances are largely accountable for the present state of confusion regarding the physiology of the gastro-intestinal canal.

Experimental data are available which indicate the myogenic nature of the rhythmic contractions of the stomach and intestine. The earlier work of Magnus, which has been quoted extensively in the literature, led him to conclude that these contractions are neurogenic. However, when he ('05) discovered that physostigmin brings out rhythmic contractions in completely denervated pieces of the intestinal musculature, he had to admit the myogenic nature of such contractions. Gunn and Underhill ('14) repeated this work of Magnus, using more refined technique, and obtained similar results. More recently, Alvarez and Mahoney ('22) presented further evidence which seems to indicate clearly the myogenic nature of the rhythmic contractions of the intestine.

If we may regard the rhythmic contractions of the stomach and intestine as myogenic, the problems regarding the activities of these organs become less baffling. The persistence of the rhythmic contractions following the administration of nicotin presents no further difficulty. Neither does it indicate that these contractions are not normally influenced by the enteric nervous mechanism. Some experimental evidence bearing on this point is already available. It is hoped that the rôle of the enteric

plexuses in the control of the rhythmic contractions of the stomach and intestine may be considered in the light of further experimental data in a later paper. On the other hand, the fact that the peristaltic contractions in the intestine, which must be regarded as neurogenic, are inhibited following the administration of nicotin, is in full accord with the discovery of synapses in the myenteric and submucous plexuses as set forth in this paper. Furthermore, all reflexes which, according to experimental evidence, are carried out within the walls of the alimentary canal are readily understood if we admit the occurrence of reflex arcs in the enteric plexuses. Therefore, the writer believes that available experimental data fully corroborate the anatomical findings set forth in this paper.

In an effort to illustrate the possible arcs mediating gastric and intestinal reflexes, the writer presented, in the earlier paper referred to above, a generalized scheme which includes afferent neurons in the myenteric plexus which send their axons centralward in the extrinsic nerves. The present study affords no evidence of such neurons. Experimental data also indicate that all gastric and intestinal reflexes which involve extrinsic nerves are carried out through the visceral afferent and efferent components of the cerebrospinal nerves. As set forth in the present paper, synapses in both the myenteric and submucous plexuses which involve two neurons located in the same ganglion suggest the occurrence of reflex arcs which involve only one or the other of these plexuses. Synapses which involve neurons in the myenteric plexus and fibers coursing in commissures which connect the latter with the submucous plexus indicate the occurrence of reflex arcs which involve both plexuses. The afferent limbs of these local reflex arcs have not been traced without interruption. Doubtless, the fibers which terminate in the gastric and intestinal epithelium are primarily the dendrites of neurons in the submucous plexus. Possibly, afferent fibers which arise in the myenteric plexus also reach the gastric and intestinal epithelium. However, the myenteric reflexes which aae stimulated by distention of a segment of the gastro-intestinal canal suggest that the afferent limbs of the myenteric reflex arcs commonly terminate within the musculature.

SUMMARY

The neurons in the enteric plexuses vary greatly in size and form and in the number, length, and distribution of their dendrites. They can probably not be classified according to two distinct morphological types. Both neurons with short, frequently branching dendrites and neurons with fewer long, infrequently branching dendrites occur in the myenteric plexus. Neurons with few relatively long dendrites preponderate in the submucous plexus.

Preganglionic fibers and fibers which arise in the submucous plexus make synapse with neurons in the myenteric plexus. Synapses involving two neurons in the same ganglion and synapses involving two neurons in separate ganglia in the same plexus occur both in the myenteric and the submucous plexus. Two neurons within the enteric plexuses which sustain a synaptic relationship to each other constitute a partial or complete local reflex arc.

The present study affords no evidence of the occurrence of nerve nets in the walls of the digestive tube.

Fibers which arise primarily in the submucous plexus terminate in the muscularis mucosae, among the glands in the mucosa, and in the gastric and intestinal epithelium. The fibers which terminate in the epithelium are probably the dendrites of neurons in the submucous plexus.

The occurrence of reflex arcs which comprise only neurons either in the myenteric or the submucous plexus as well as those which comprise neurons in both plexuses is indicated by the anatomical findings. Consequently, a structural basis is afforded for the rational interpretation of all the usual activities of the stomach and intestine except the rhythmic contractions. Attention is called to the work of Magnus, Gunn and Underhill, and Alvarez and Mahoney which indicates the myogenic nature of these contractions.

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Resumen por el autor, E. A. Boyden.

El desarrollo temprano de la cloaca en los embriones del avestruz, con especial mención de la reducción del intestino caudal.

El presente trabajo es el primero de una serie de articulos sobre la embriología del avestruz. En él se sigue el desarrollo de la cloaca hasta el estado de 18 mm.—esto es, hasta la aparición de la bolsa de Fabricio. Su interés principal está en la explicación que ofrece de la fenestra cloacal—una estructura descrita recientemente por el autor en los embriones de pato, faisán y gallina. El trabajo se ocupa también del seno urodeal, una repetición embrionaria de la vejiga dorsal de los saurios, el cual deriva, en el avestruz, del extremo proximal del intestino caudal. Dentro del periodo estudiado no existe oclusión del recto, pero el colon exhibe asas que le distinguen del de la mayor parte de las otras aves. En general, la cloaca del avestruz se parece más a la cloaca de los embriones jóvenes de reptiles que a la de cualquier otra ave estudiada hasta el presente.

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THE EARLY DEVELOPMENT OF THE CLOACA IN OSTRICH EMBRYOS, WITH SPECIAL REFERENCE TO THE REDUCTION OF THE CAUDAL INTESTINE

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ONE PLATE (EIGHT FIGURES)

The cloaca of the ostrich presents two characteristics of unusual interest, the enormous size of the male copulatory organ and the persistence, throughout adult life, of an undiminished cloacal bursa (bursa Fabricii). As early as 1836, the first of these peculiarities attracted the attention of Johannes Müller, who described two types of erectile organs found in ratite birds and appended to this account a discussion of the developing form of copulatory organs in vertebrates in general. The second feature attained significance, somewhat later, through the investigations of Forbes, Wenckebach, and Gadow. These men demonstrated not only the persistence of the bursa in adult Ratitae, in contrast to its early atrophy in the young of carinate birds, but also its great size and the peculiar function it subserves as a urinary bladder.

Since the bursa of the ostrich differs so markedly from the corresponding organ in other groups, it occurred to me that a careful study of its development and histogenesis might throw some light on the significance of the bursa in flying birds, in which forms it has been variously interpreted as a lymphoid structure, a gland of internal secretion, a vestigial organ of obscure antecedents, etc. An opportunity to secure a graded series of embryos for this purpose was provided unexpectedly by a trip to California. It is a great pleasure, in this connection, to acknowledge the hearty cooperation of the manager and attendants of the Cawston Ostrich Farm at South Pasadena and the courtesy of Prof. A. W. Meyer, of Stanford University, who provided the reagents used in collecting the embryos.

The present paper is in the nature of a preliminary report and is restricted to a consideration of the early development of the cloaca, up to the stage ending with the appearance of the bursa of Fabricius. Its chief interest lies in the bearing which it has on the cloacal fenestra, a structure recently discovered by the author in embryos of the common fowl, duck, and pheasant.[1]

This fenestra was described as a temporary foramen, caused by the disintegration and subsequent removal of that portion of the cloacal wall subjacent to the dorsal aorta. It was held to be of special interest not merely because it furnished the only instance, in the differentiation of a hollow organ, in which a gap occurs in the epithelial wall as a normal and constant feature of development, but also because it provided landmarks which established for the first time the exact point of origin of the bursa of Fabricius.

The phenomenon was thought to be intimately associated with the retrograde process causing the atrophy of the caudal intestine, since the area of disintegration spread posteriorly into the caudal intestine soon after it appeared on the flanks of the cloaca (see vertically lined area in figure 4). This interpretation has been greatly strengthened by the series of ostrich embryos presented in figures 1 and 2 and 5 to 8, as well as by comparison with the development of the caudal intestine in turtle embryos.

To make the comparisons clear it is necessary to review the initial stage of fenestra formation indicated in figure 4. This is a reconstruction of a 41-somite chick embryo in which the ends of the Wolffian ducts are about to fuse with the cloaca. The caudal intestine is patent and its cavity extends from cloaca to tail-bud. The angle subtended by the cloaca and the caudal intestine (see area marked by crosses) contains a dense mass of undifferentiated tissue which, like the tail-bud, is a persistence of the primitive streak of earlier stages.[2] The epithelium

[1] The development of the cloaca in birds, with special reference to the origin of the bursa of Fabricius, the formation of a urodaeal sinus, and the regular occurrence of a cloacal fenestra. Am. Jour. Anat., vol. 30, no. 2, March, 1922.

[2] The exact interpretation of this tissue is subject to reservation and awaits further investigation. The author has followed Gasser in deriving this from the primitive streak, with which it is admittedly continuous from its earliest appearance.

bordering this mass (that is, the inner curvature of the caudal intestine) and the wall of the cloaca adjacent to the anal plate are not fully differentiated from the primitive streak. The vertical lines in the figure indicate paired epithelial areas on the flanks of the cloaca which are about to disintegrate. The subsequent removal of these and adjoining areas by phagocytosis severs the caudal intestine from the cloaca, and exposes the contents of the latter to the mesenchyma through a broad fenestra. (For further details see publication referred to on page 212.) In the duck the cavity of the caudal intestine is occluded at a distance from the cloaca (cf. x in fig. 3), but does not rupture before the fenestra develops, whereas in the tern the tube becomes solid and ruptures at the point of occlusion, without the occurrence or intervention of a fenestra.

It is now appropriate to consider the reduction of the caudal intestine in turtle embryos. Figure 3 is a reconstruction of a 45-somite Chrysemys embryo slightly older, relatively, than the chick shown in figure 4. The caudal intestine has ruptured at x and its cavity on each side of the break is occluded. The primitive-streak mass has been reduced to a thin plate appended to the inner curvature of the caudal intestine. The most interesting feature is the method by which the cloacal end of the tube undergoes reduction. The vertically lined area in the figure marks the position of an external groove which corresponds to a longitudinal fusion of the lateral walls inside and a consequent obliteration of the cavity at this level. The effect of this fusion is two-fold. It has reduced the broad opening of the caudal intestine to a narrow passage in the lower segment of the cloaca and it has shifted its outlet forward as far as the level of the Wolffian ducts. The continuation of this process in later stages reduces even the lower segment to a solid plate of disintegrating tissue. The grooved area in figure 3 is undergoing phagocytosis and corresponds in position to the disintegrating area in the walls of the chick, which there becomes modified into a cloacal fenestra. And it accomplished the same result, namely, the obliteration of the proximal portion of the caudal intestine.

A third method of reducing the caudal intestine is exhibited by ostrich embryos. The first stage is shown in figure 1, of an embryo slightly older than the chick, but somewhat younger than the turtle embryo just described. The primitive-streak tissue has become separated from the anal wall of the cloaca, but still forms the inner curvature of the caudal intestine. As in the turtle, this tissue has been reduced to a thin plate one or two cells thick. It may be distinguished from the mesenchyma of the tail by its deeper stain and its homogeneous appearance. The once continuous lumen of the caudal intestine has been broken up into isolated cavities by the irregular adhesion of its walls, this process taking place in such a way as to give the cavities that remain a wave-like contour.

In the next stage, figure 2, the lumen of the caudal intestine is entirely obliterated and forms, with the primitive streak, a ribbon-like band of tissue running through the core of the tail. In the youngest portion of the caudal intestine, near the end of the tail, the lumen still persists and both caudal intestine and primitive streak, as in figure 1, merge with the tail-bud.

In the third stage, represented by figures 5 and 6, the caudal intestine has ruptured from the cloaca, and its distal end, except that portion in contact with the tail-bud, has disappeared. The proximal end is still attached to the cloaca, but its outlet has been shifted as far forward as the Wolffian duct. Whether this takes place by such a progressive adhesion of lateral walls as occurs in the turtle, or is accompanied by the breaking through of an adhesion, such as occurs at the point indicated by an asterisk in figure 5, is uncertain, since two of the available embryos of this age were like figure 5 and two were like figure 6. While the first type may be anomalous and may represent merely a delayed fusion of the walls in the region behind the break, it is quite possible that figure 5 is the step which normally precedes the stage shown in figure 6.

There are thus presented, in the Sauropsida, three modifications of a method by which the cloacal end of the caudal intestine is reduced. In turtles the projecting stump of the tube is constricted off by a progressive adhesion of the lateral walls of the

cloaca. In the ostrich, in at least two cases, the constriction breaks through in the middle after the manner in which the semicircular canals of the ear are cut out of the otocyst. In the duck and gallinaceous birds the corresponding areas of the cloacal wall disintegrate before adhering, thereby producing a temporary lesion known as the cloacal fenestra. It is significant that the most conspicuous of these methods, that found in the chick, is associated with the persistence of the greatest amount of undifferentiated primitive streak and that in birds like the tern, in which none of the undifferentiated tissue remains, the caudal intestine ruptures at the proximal end and is absorbed into the cloaca as uneventfully as in mammals.

The ultimate disposition of the cloacal end of the caudal intestine in the ostrich is very surprising. A comparison of figures 6, 7, and 8 shows that the blunt end of the tube becomes converted into a permanent diverticulum (diverticulum 'c' of figure 8) which corresponds in position to the urodaeal sinus of the chick embryo, a structure which was interpreted to be a repetition of the dorsal bladder found in snakes and lizards. In the domestic fowl this diverticulum arose as a new outpocketing of the dorsal wall between the openings of the two Wolffian ducts, soon after the fenestra had severed the outlet of the caudal intestine from the cloaca. Its mode of origin in the ostrich and tern embryos suggests that the urodaeal sinus of all birds is primarily a derivative of the cloacal end of the caudal intestine.

The ostrich cloaca also suggests a possible explanation for the two accessory diverticula in the chick, defined as diverticulum 'a' and 'b.' When these were first seen it was thought that they were irregularities left at either end of the fenestra by that destructive process, but a study of the ostrich series shows that diverticulum 'a' is a constant feature in a bird which never develops a cloacal fenestra. It appears in stages represented by figures 5 to 7, and either becomes incorporated in the bursa or disappears before the latter is formed. In the chick it happens to lie at the caudal boundary of the fenestra, and this incidental relation may explain its larger size in that embryo. But in both birds it is a temporary structure, and the only definite thing

about its position is that it arises at the point where the primitive streak originally joined the cloaca.

Diverticulum 'b,' on the other hand, is not found in the ostrich, and only occasionally in the chick, where it appears at the cephalic end of the fenestra and always adjacent to the urodaeal sinus (figs. 24, 28, 30, and 32 of the previous paper). This double relation, in view of the origin of the sinus in the ostrich, suggests that diverticulum 'b' in the chick may represent a remainder of the cloacal end of the caudal intestine left by the formation of an incomplete fenestra. This, in turn, raises the question as to whether the urodaeal sinus of the ostrich may not be of double origin, arising chiefly from the remainder of the caudal intestine, but also in part from a possible, independent outpocketing of the cloacal wall comparable to diverticulum 'c' of young chick embryos. In figures 5 and 7 such a diverticulum is barely suggested in the region between the caudal intestine and the rectum. The issue raised, however, is a fine one and does not jeopardize the statement that the bulk of the urodaeal sinus is derived from the cloacal end of the caudal intestine.

Another interesting feature of this region in the ostrich is the coiling of the large intestine, the beginning of which process can be seen in figures 7 and 8. In most other birds the large intestine remains a short straight segment of the gut, leading from the intestinal caeca to the cloaca. But in Struthio, as Owen pointed out long ago, the large intestine is much coiled and twice as long as the small intestine. In view of this fact, Owen's use of the term rectum to include the whole length of the large intestine in birds seems ill advised; and it is to be questioned whether Gadow's statement that the colon is present only in Struthio can be justified.

The unusual size of the intra-embryonic portion of the allantois also merits attention. This structure in the chick was described as an exact duplication of the dilated area in reptile embryos which develops into the ventral bladder of the adult. In the ostrich it is even more conspicuous than in the chick, having a recurrent lobe (fig. 8, *all. bl.*) which fills up the posterior end of the body cavity. But in both species it disappears before hatching.

Two other respects in which the ostrich differs from the chick are the failure of the rectum to become occluded before the 18-mm. stage is reached and the modification of the method by which the urodaeal membrane is formed. The combination of these two processes in the chick restricts the cavity of the cloaca to a narrow channel connecting the Wolffian ducts with the allantois, the alleged purpose of which, as suggested by Parker, is "to prevent the escape of the excretion either into the intestine or into the amniotic cavity, where it might prove injurious to the embryo." Although the occlusion of both regions is completed by the 15-mm. stage in the chick, there is no narrowing of the lumen in the rectum of an 18-mm. ostrich, while the urodaeal membrane is barely indicated by the approximation of the flanks of the cloaca (dotted area, fig. 8). Yet in this same embryo the metanephros is as far along as in a 15-mm. chick. It is not improbable that the examination of older embryos will show that there is no solid stage to the large intestine in the ostrich.

The peculiarity of the urodaeal membrane in the ostrich lies in the order of its formation. In the chick the lateral walls of the cloaca first meet in the region of the anal plate, then along the caudal margin of the cloaca. From these two places the area of fusion spreads anteriorly toward the allantois and rectum (cf. plate 3 of article referred to on page 212), until all that portion of the cavity has become obliterated and transformed into a flat membrane. In the ostrich (dotted area, figs. 6 to 8) it begins at the anal plate and extends to that part of the caudal wall adjoining the urodaeal sinus, thereby leaving a deep pocket adjacent to the anal plate. This has an important bearing on the origin of the bursa, because in all other birds studied the bursa originates from a proliferation of that portion of the caudal margin nearest the anal plate. But in figure 8 the primordium of the bursa (indicated by three big vacuoles in the lateral wall of the cloaca as well as by the thickened caudal margin) is located as near to the urodaeal sinus as it is to the anal plate. It is quite probable that the undotted area near the anal plate is merely the last portion of the cavity to be reduced, but the reversal of order

is significant as being the first difference noted between the development of the bursa in carinate and ratite birds. It would seem to indicate that the bursa of the ostrich will occupy all the margin of the cloaca between the urodael sinus and the anal plate, thereby explaining the unusually wide orifice of the bursa in the adult bird which, it is said, partially houses the retracted penis.

The early development of the cloaca in the ostrich thus offers many interesting points of comparison with the cloaca of other bird embryos. And, in general, it exhibits a distinctly more reptilian character than the cloaca of carinate embryos—in the method by which the caudal intestine is reduced, in the position of the Wolffian ducts, in the size of the allantoic bladder, in the very thin anal plate, in the elongation of the large intestine, and in length of tail and number of caudal somites. It is such primitive characters as these which arouse the hope that further study of this series of embryos will contribute much to an understanding of the significance of the bursa of Fabricius and the origin, in general, of avian from reptilian structure.

PLATE

PLATE 1

EXPLANATION OF FIGURES

Graphic reconstruction of sauropsidan embryos drawn to the same scale. × 35. This plate should be compared with plates on pages 167 and 199, Am. Jour. Anat., vol. 30, 1922. Dash lines indicate cavities; dotted lines, vacuoles or somites; dotted areas, regions in which opposite walls of the cloaca are nearly in contact; areas with crosses, primitive-streak remainders; vertically lined areas, areas of disintegration. *a* represents a constant, though temporary diverticulum homologous with diverticulum *a* of chick embryos. *c* represents a diverticulum which regularly forms the medial component of the urodaeal sinus: in ostrich embryos it is derived from the proximal end of the caudal intestine.

1 Ostrich embryo (Struthio australis[1]), H.E.C. 2234. 56 somites.
2 Ostrich embryo, H.E.C. 2235. 7 mm.
3 Turtle embryo (Chrysemys marginata), H.E.C. 1067. 6 mm. (after R. F. Shaner).
4 Chick embryo, H.E.C. 2071. 41 somites. 3 days, 18 hours.
5 Ostrich embryo, H.E.C. 2239. 10.2 mm.
6 Ostrich embryo, H.E.C. 2240. 10 mm.
7 Ostrich embryo, H.E.C. 2243. 13.5 mm.
8 Ostrich embryo, H.E.C. 2245. 18 mm.

ABBREVIATIONS

a, diverticulum 'a'
all., allantois
all.bl., allantoic bladder
an., anal plate
*(asterisk), area occupied by mesenchyma
bursa., bursa cloacae (Fabricii)
c., diverticulum 'c' (urodaeal sinus)
c.i., caudal intestine
col., large intestine
n.t., neural tube
pelv., pelvis of kidney
pr., proctodaeum
p.s., primitive-streak remainder
rect., rectum
umb., constriction of pelvis caused by umbilical artery
ur., ureter
ur.m., urodaeal membrane
W.d., Wolffian duct
x., rupture of the caudal intestine

[1] All the ostriches figured on this plate are probably Struthio australis, the South African species. There is a slight possibility that the specimens contain a strain of the big Nubian ostrich, Struthio camelus.

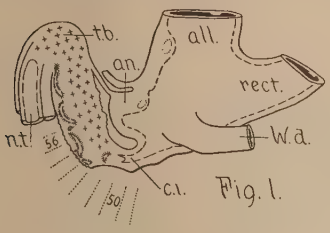

Fig. 1.

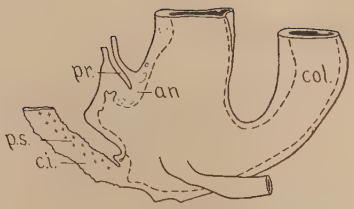

Fig. 2

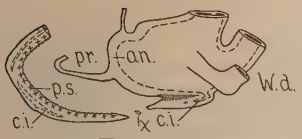

Fig. 3.

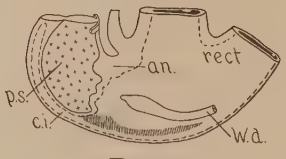

Fig. 4

Fig. 5.

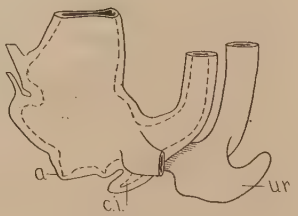

Fig. 6

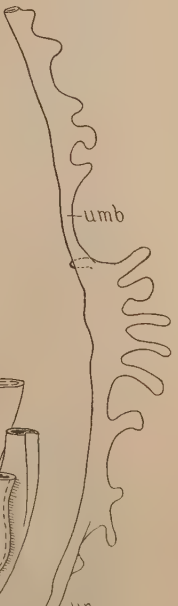

Fig. 7.

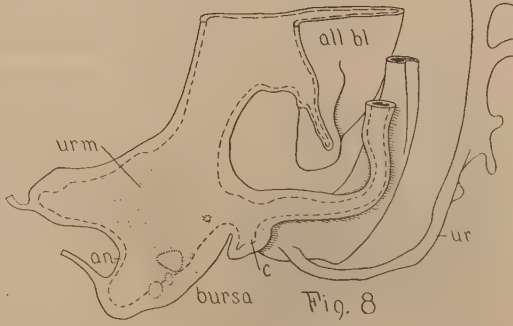

Fig. 8

Resumen por los autores, P. N. Johnstone y F. H. Wakefield.

Sobre el carácter de las fibras de Purkinje en varias regiones del fascículo atrio-ventricular.

La presente investigación se ocupa de la morfología de las fibras de Purkinje en varias regiones del fascículo atrio-ventricular. El método empleado consiste en cortar en serie bloques de tejido tomados de las regiones objeto de estudio, reconstruyendo las fibras de Purkinje. Con este objeto los autores han empleado el método de Born mediante placas de cera y el método consistente en la superposición y combinación de cortes seriados dibujados en papel. Los autores han llegado a las siguientes conclusiones mediante el estudio de otros cortes y las reconstrucciones: 1) Las fibras de Purkinje del nodo atrio-ventricular, conforme ha demostrado Tawara, son mucho más pequeñas y se ramifican y anastomosan más extensamente que en cualquier otra región del fascículo atrio-ventricular. 2) Las fibras de Purkinje del tronco y de los miembros derecho e izquierdo del fascículo son muy semejantes; las del tronco son más sinuosas. En corte transverso presentan una forma algo cilíndrica. Aunque son mayores que las del nodo, parecen mucho más pequeñas que en las expansiones terminales. Se ramifican y anastomosan menos que las fibras del nodo, pero más que las de las expansiones terminales. 3) Las fibras de Purkinje de las expansiones terminales son polimórficas. Hay dos tipos extremos: el de forma de bastón y el foliáceo. En nuestra reconstrucción de las fibras mencionadas del músculo papilar posterior del ventrículo izquierdo la fibra foliácea está algo fenestrada. 4) La acumulación de tejido de Purkinje en las expansiones terminales es mucho mayor que en el tronco y miembros del fascículo. 5) Los autores no han encontrado porciones aisladas del tejido de Purkinje.

Translation by José F. Nonidez
Cornell Medical College, New York.

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
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ON THE CHARACTER OF THE PURKINJE FIBERS IN VARIOUS REGIONS OF THE ATRIO-VENTRICULAR BUNDLE

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SIX FIGURES

Purkinje, in 1845, first called attention to the presence in the subendocardial layer of the sheep's heart of a system of fibers that are of a different character from heart muscle. These fibers are found in the ventricles ramifying over their surfaces. Numerous writers have since pointed out, along with Purkinje, that they are to be found in the hearts of many other animals including the pig, calf, goat, horse, dog, cat, rat, mouse, goose, hen, dove, and others. They are usually situated immediately under the endocardium, but are at some places to be found in the myocardium. Hoffman has given evidence that they are to be found in the pericardium. If the left ventricle of a fresh sheep's heart is opened the fibers that were first described by Purkinje will appear as glistening thread-like strands. They are present in almost all the regions of the ventricle, but are noticeably absent in certain regions, including that portion of the ventricular septum immediately beneath the septal cusp of the aortic valve. They are to be found in great abundance in the regions of the trabeculae carneae where they seem to pass as single strands from trabecula to trabecula. They are seen in considerable quantities at the bases of the papillary muscles and in some instances find their way almost to the apices. As seen macroscopically on the inner surface of the ventricle, they are found to branch and anastomose quite extensively, forming a most intricate network.

W. His, Jr., in 1893, discovered a bundle of fibers, which he thought to be muscle, connecting the right atrium with the

ventricles. This bundle of fibers known as the atrioventricular bundle, so named by His, arises in the right atrium from a node known as the atrioventricular node. This node was discovered by Tawara and is described in his monograph. It lies beneath the coronary sinus of the right atrium and approaches the atrial septum more nearly than does the coronary sinus. The atrioventricular bundle continuing from the node travels in the general direction of the ventricular septum. Upon reaching the septum it divides into a right and a left limb. In the calf's heart the right limb is at first subendocardial, but as the septum thickens it becomes imbedded to a depth of several millimeters in the heart muscle of the ventricular septum. The left limb is found immediately beneath the endocardium. After traversing a short distance, the left limb bifurcates into an anterior and a posterior part, while the right limb continues undivided. In his investigations Tawara concluded that the fibres which were described by Purkinje were merely continuations of the atrioventricular bundle. Lydia DeWitt conclusively proved that this was the case by dissecting the entire structure and then modeling it to scale.

All of the fibers of the sinoventricular system—though differing greatly in size, shape, and arrangement in its various regions —because they are composed of a related if not an identical tissue will hereafter in this report be called Purkinje fibers. The tissue composing the Purkinje fibers will be termed Purkinje tissue. Tawara in his monograph has divided the atrioventricular bundle into two main parts, the atrial part and the ventricular part. He subdivides the atrial part into the node and the trunk, and the ventricular part into the upper undivided part and the terminal expansions, or the Purkinje fibers. In this report we shall employ Tawara's classification in the main. We shall not use the terms 'terminal expansions' and 'Purkinje fibers' synonymously. For the purpose of clearness, we have outlined the nomenclature that is employed in this report (fig. 1).

Our method of studying the Purkinje fibers in the various regions of the atrioventricular bundle consisted essentially of making serial sections of the fibers from the regions to be studied

and of reconstructing them. All of the sections were taken from one calf heart. Akey gives a diagrammatic representation of the atrioventricular bundle representing the regions from which the reconstructions were made. The heart was procured

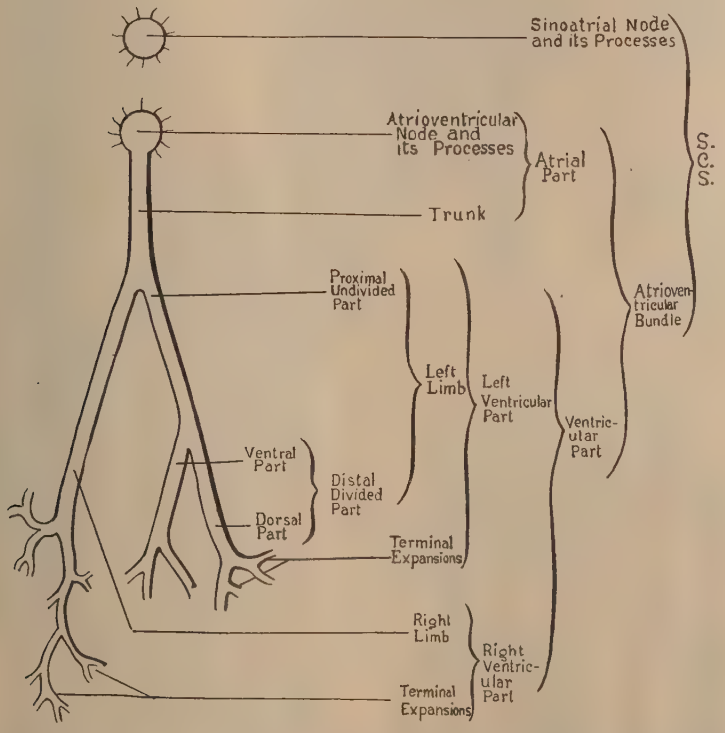

Fig. 1 An outline of the nomenclature used in this paper. Purkinje fibers: all of the fibers of the sinoventricular conducting system that are composed of Purkinje tissue. S. C. S. = sinoventricular conducting system.

immediately after the animal was killed. It was carried to the laboratory and there opened in the following manner: An incision was made along the marginal surface of the left ventricle and atrium. With the scissors the aorta was opened along a line drawn through the left sinus of Valsalva and the apex of the left

Fig.2
Fig 4
Fig.3
Fig.5
Fig.6
2
3
4

Figs. 2 to 6 Drawings of reconstructions of various parts of the atrioventricular bundle of the calf's heart. All five were taken from the same heart, fixed in Carnoy's fluid, imbedded in paraffin, sectioned at 25 μ, and stained with hematoxylin and eosin. All are reconstructed at the same magnification of 73.2 diameters, reduced one-third in reproduction, to a magnification of 48.8 diameters. Only Purkinje tissue is reconstructed.

The reconstructions shown in figures 2 to 4 were made by superimposing and combining serial sections on paper. Those shown in figures 5 and 6 were made by the Born wax-plate method. Figure 2 is from the trunk. Figure 3 is from the right limb. Figure 4 is from the left limb. Figure 5 is from a moderator band of the left ventricle. Figure 6 is from the posterior papillary muscle of the left ventricle.

ventricle. The anterior surfaces of the pulmonary artery, the conus arteriosus, and the right ventricle were cut through with the scissors. When the apex of the ventricle was reached the cut was turned directly along the marginal surface of the right ventricle, through the atrioventricular junction and along the marginal surface of the right atrium. This exposed the atrioventricular bundle without damaging it. Blocks of tissue were taken from it in the following regions: 1) the trunk, 2) the right limb, 3) the proximal undivided part of the left limb, 4) a moderator band of the left ventricle, 5) the posterior papillary muscle of the left ventricle. The blocks were all made large where possible so as to avoid any possible distortion of the Purkinje fibers due to curling. They were then fixed in Carnoy's solution, imbedded in paraffin, and sectioned at a thickness of 25 μ. In all cases the Purkinje fibers were cut longitudinally. The sections were stained with hematoxylin and eosin and were mounted in balsam-damar.

No difficulty was encountered in making the reconstructions of the Purkinje fibers of the moderator band and of the papillary muscle by the Born wax-plate method. The Purkinje fibers of the trunk, the right limb, and the left limb were reconstructed by the method of superimposing and combining successive sections on paper. It was found that they were too small at the magnification planned to admit of accurate results by the wax-reconstruction method. The reconstructions were all done at a magnification of 73.2 diameters.

In his monograph Tawara has shown very good illustrations of the Purkinje fibers of the atrioventricular node which he described. Our sections have only confirmed the findings of Tawara, that the Purkinje fibers of the atrioventricular node are very much smaller and very much more tortuous than are the fibers of the trunk. Our reconstructions of the Purkinje fibers of the trunk show that they have a gnarled arrangement and that they branch and anastomose quite extensively (fig. 2). The Purkinje fibers of the right and left limb are virtually identical and are less gnarled than are those of the trunk (figs. 3 and 4). The size of the Purkinje fibers of the two limbs in com-

parison with those of the trunk is about the same. Individual Purkinje fibers in either of these three regions vary in size. In cross-sections made from other hearts the Purkinje fibers of the trunk and of the limbs appear to be somewhat cylindrical in shape.

The reconstruction of the Purkinje fibers of the moderator band shows the fibers to be very much larger, and that very much less branching and anastomosing takes place than in either the limbs or the trunk (fig. 5). Moreover, the Purkinje fibers seem to be of two kinds in this reconstruction, one being more cylindrical in shape and the other of the character of a sheet. The Purkinje fibers in the reconstruction of the moderator band that are more rod-like in shape are many times as large as the fibers in the limbs and the trunk. The Purkinje fiber having the character of a sheet in this reconstruction is very broad. It is nearly as broad as all the other Purkinje fibers of the moderator band. It is not fenestrated. One of the more rod-like Purkinje fibers is hollow throughout a part of its course. This tubular formation is finally terminated at a mouth which opens in the side of the fiber.

The Purkinje fibers which were reconstructed from the papillary muscle show a broad sheet-like fiber which is somewhat fenestrated, along with several instances of rod-like fibers (fig. 6). These are apparently continuations of the corresponding types seen in the moderator band. Our reconstruction, along with a study of sections from other hearts, leads us to believe that these fibers are polymorphous. These shapes we believe to be bounded by the rod-like and the sheet-like fibers as the two extremes. The sheet-like fiber of the papillary muscle is the largest fiber in our reconstructions. The increase in the size of the Purkinje fibers in the terminal expansions is typical, as examination of sections from other hearts shows this characteristic. As isolated portions of Purkinje tissue were not found in any of the reconstructions, it is reasonable to suppose that they do not exist.

SUMMARY

In this investigation the Purkinje fibers in various regions of the atrioventricular bundle were studied by reconstruction methods. It has been found that—

1. The Purkinje fibers of the atrioventricular node, as was shown by Tawara, are very much smaller and branch and anastomose more extensively than in any other region of the atrioventricular bundle.

2. The Purkinje fibers of the trunk and of the right and left limbs are very similar, the fibers of the trunk being more gnarled. In cross-section they seem to have a somewhat cylindrical shape. Though they are larger than in the atrioventricular node they are much smaller than in the terminal expansions. They branch and anastomose less than the Purkinje fibers in the atrioventricular node but more than the Purkinje fibers in the terminal expansions.

3. The Purkinje fibers of the terminal expansions are polymorphous. There are two extreme types, the rod-like and the sheet-like. In the reconstruction of the Purkinje fibers of the moderator band of the left ventricle the sheet-like fiber is not fenestrated, but in the reconstruction of the Purkinje fibers of the posterior papillary muscle of the left ventricle the sheet-like fiber is somewhat fenestrated.

4. The accumulation of Purkinje tissue in the terminal expansions is far greater than in the trunk or in the limbs.

5. Isolated portions of Purkinje tissue were not found.

In conclusion, we wish to express our appreciation to Dr. E. R. Clark for the assistance that he has rendered in the carrying out of this investigation. We wish also to acknowledge the services of Mr. G. T. Kline, who made the final drawings of the illustrations.

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Resumen por el autor, John S. Latta.

La interpretación de los llamados centros germinales del tejido linfático del bazo.

Desde la publicación de los resultados de la investigaciones de Flemming ('85) en los cuales dicho autor concluye que las áreas centrales más claras de los nódulos linfáticos densos son centros de proliferación, o "centros germinales," los investigadores que han estudiado el problema del tejido linfático han estado acordes invariablemente con este concepto de su significación. En un estudio reciente ('21) de estas estructuras en el tejido tonsilar intestinal, no he encontrado prueba alguna de la actividad proliferativa de estas áreas, sino que he llegado más bien a la conclusión de que representan centros de degeneración. Un cuidadoso estudio histogenético ha sido llevado a cabo por el autor en los centros de los nódulos linfáticos esplénicos. En estas regiones no he podido hallar prueba alguna de actividad proliferativa o transformativa. El aspecto más claro de estos centros, cuando existen, se debe a una menor densidad de los linfocitos libres en este sitio, asociada con mayor prominencia del tejido reticular. El autor ha encontrado estados de la degeneración de los pequeños linfocitos y hemoblastos linfoides, los cuales se deben, sin duda alguna, a la disminución de la nutrición de los centros. El autor indica, por consiguiente, que el término "centro germinal" no describe las condiciones existentes en estos puntos, y que deben considerarse más bien como centros en los cuales tienen lugar cambios degenerativos.

Translation by José F. Nonidez
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THE INTERPRETATION OF THE SO-CALLED GERMINAL CENTERS IN THE LYMPHATIC TISSUE OF THE SPLEEN

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THREE FIGURES

In the histological examination of dense lymphatic tissue as found in the lymphatic nodes, the various tonsils, and in the spleen of mammalian forms, it is noted that often the central portions of the lymphatic nodules are of lighter appearance than their peripheral portions. This fact was first studied in detail by Flemming ('85), who designated these lighter central portions of nodules, 'Keimzentrum,' or germinal centers, for he considered these areas of the nodules as centers of proliferation of lymphocytes. Since that time investigators have invariably followed Flemming in his interpretation of these light colored areas. There has been, however, apparently some confusion as to exactly what is meant by the term, germinal center, for several authors have included in this term an entire lymphatic nodule (Baum u. Hille, '08; Bunting, '05), instead of their lighter colored central portions, as evidently intended by Flemming and most investigators following him.

As I have indicated in a previous paper ('21), in the study of the lymphatic tissue of the intestinal tonsils, it seemed that the term, germinal center, was non-descriptive of conditions existing in those nodules of the intestinal wall which contained these light colored central areas. Results of this study seemed to indicate that the so-called 'germinal center' was not a place of special lymphocytic formation and proliferation, but rather one of degenerative changes.

Whether these centers are proliferative or degenerative in character, their interpretation is very closely associated with the nodular blood supply (i.e., the question of nutrition). In this respect, the Malpighian bodies (lymphatic nodules) of the spleen are of special interest because of the fact that they here develop as an hypertrophy of portions of the lymphoidal arterial sheaths, thereby changing, to some extent, the usual vascular relationships of nodules in lymphatic tissue. Then, too, the histological appearance of splenic nodules which possess the so-called 'germinal centers' seems to differ somewhat from nodules in other varieties of lymphatic tissue. Therefore, it is thought, that some study of the splenic lymphatic tissue, with special reference to the presence of the lighter colored central areas, their relation to the vascular supply, and their histological structure and development, might aid in more clearly interpreting their significance.

Human spleens, collected from autopsies performed at the University Hospital, University of Nebraska, in which the causes of death were, in no apparent way, associated with the spleen, were used chiefly in this investigation. These were from individuals of ages varying from still-born infants, in which the splenic nodules were ill-defined and few in number, to adults of somewhat advanced age. This human material was supplemented by spleens of dogs, cats, guinea-pigs, and rabbits, which were used for comparative purposes.

The fixing fluid found to give the best results with the stains which followed, and hence that used most extensively, was Helly's fluid or Zenker-formalin, prepared by adding 10 per cent of formalin to Zenker's stock solution. Picro-aceto-formalin (Bouin's fluid) was also used to some extent with very good results.

All of the material was imbedded in paraffin, the sections cut from 6 to 8 μ thick, and portions mounted serially, to be sure that correct relationships between the nodules and the surrounding parts could be determined.

The most commonly used stains were the blood stains, Hastings-Nochts modification of the Romanowski blood stain, and a

½ per cent solution of alcoholic eosin in methyl alcohol, followed by an aqueous solution of methylene blue, made slightly alkaline with KOH (as used by Gage). A solution composed of equal parts of 1 per cent aqueous solutions of methyl green and pyronin gave good results (after Pappenheim). Finally Mallory's aniline-blue connective-tissue stain was used, mainly upon tissue which had been previously fixed in picro-aceto-formalin, to show clearly the extent and amount of the reticular tissue in its relation to the splenic nodules.

In 1854, Brücke, in a study of lymphatic tissue, noticed that occasionally the dense nodules of this tissue had lighter colored central areas. He said, "One sees occasionally in the cortical nodules and still more often in Peyer's patches a central lighter portion." His ('62) also had observed lighter areas in the center of lymphatic nodular masses, which he called 'Vacuolen.' Frey ('74) said, "In the large follicular masses are seen lighter colored translucent centers." These centers, he considered, were the true follicles, the darker periphery being something peculiar to these follicles.

Flemming ('85) made a more exhaustive study of lymphatic tissue than previous investigators. He used as objects of study mesenteric lymphatic nodes from the ox and rabbit, Peyer's patches from the caecum of rabbits, and human lingual tonsils. He observed these lighter central areas in all of these different tissues, and considered them to be of the same significance in the tonsils, lymphatic nodes, and the spleen. In the center were cells with a large amount of cytoplasm and large nuclei, while cells at the periphery were smaller and stained more deeply, thus giving a lighter appearance to the central portion. In the center mitotic figures were prominent and at the periphery were scarce. Consequently he called this lighter central area the 'germinal center.'

Subsequently, as has been before intimated, there has been some confusion in regard to the 'germinal center,' and to what the term should be applied. Leading text-books have invariably followed Flemming in their interpretation of this structure, but in the study of the results of investigators of problems relating

to lymphatic tissue, one finds that the term 'germinal center' is often used in an indefinite way. The use of the term by Bunting ('04) and Baum and Hille ('08) has already been referred to. Gulland ('94) also seems to have had a rather vague idea of the significance of this term. He says the germinal centers (in lymphatic tissue) are known as light circles with dark circumferences. Sabin ('05) considers the second part in the development of lymph nodes to be the formation of germ centers, which latter she described as condensations of cells where lymphocytes are actively proliferating (also Ribbert, '89; Richter, '02).

These descriptions seem unsatisfactory as concerning the so-called 'germinal centers,' but correspond rather with current conceptions of complete dense lymphatic nodules.

Czermack ('93) followed Flemming in believing the 'germ centers' of lymphatic nodules to be composed of cells which continually produced lymphocytes. These cells he called 'Keimzellen.'

Saxer ('96), in speaking of 'germ centers,' said that the presence of more numerous divisions of fixed cells in the germ centers than in reticular and areolar tissue in other places is understandable because of the increased physiological function and the importation of nutritive elements. Just what he included under the term 'Keimzentrum' could not be determined.

All recent investigators who have studied the 'germinal centers' of lymphatic tissue agree with Flemming in the general interpretation of their significance (Schridde, '07; Maximow, '09; Downey and Weidenreich, '12; Hartmann, '14; Thiel and Downey, '21; Nakahara and Murphy, '21). The lymphoblastic nature of cells in the 'germinal center' has been quite generally accepted. Some authors have followed Czermack in giving these cells the specific name of 'germinal center cells' (as Hartmann, '14). Thiel and Downey, in discussing the germinal centers of the spleen, considered these areas as regions of special activity of the reticular tissue, as manifested by the hypertrophy, increase in number, isolation, and transformation of its cells.

In addition to cells of a lymphoblastic character, there have been demonstrated in the light colored 'germinal centers' of lymphatic tissue in various places certain large phagocytic cells (those containing the 'tingible Körper' of Flemming), considered by some to be derived from cells of the reticular tissue (Weidenreich and Downey, '12; Hartmann, '14), and, as studied by myself in intestinal tonsillar tissue ('21), thought to be derived by further differentiation and degeneration of lymphoid hemoblasts.

As the special proliferative activity of the 'germinal centers' is accounted for by many as a result of increased physiological function and unusual possibilities of nutrition (Saxer, Thiel and Downey, et al.), and as my previous explanation of them as degenerative centers is based upon an insufficient blood supply at these points, the relation of the lymphatic nodules to the vascular supply was a question of special interest in the present investigation.

Although, as before stated, splenic tissue from the dog, cat, and other forms were studied for comparison with the structure of the human spleen, in all respects in which comparison was made there was little or no variation, so the conclusions arrived at in this paper are based upon the study of the human spleen.

It becomes very evident, when sections of the spleen from individuals of varying ages are studied, that arteries of the spleen very early (before birth) acquire lymphoidal sheaths (which, according to Danchakoff, Downey, and others, is preceded by a mesenchymatous sheath). At the time of birth, however, there is little distinction to be drawn between the red and the white pulp (lymphatic tissue). The lymphoidal arterial sheaths, however, seem to increase markedly in density, and, in young adults, are a very distinctive splenic structure. Lymphatic tissue was very abundant in all of the adult human spleens studied, more particularly in their central portions.

Malpighian bodies, or splenic lymphatic nodules, are not recognized until some time after the formation of the lymphoidal arterial sheaths. They are formed, according to Downey and Weidenreich ('12), by the rapid increase of the number of lymphocytes, derived from fixed cells of the reticulum, in certain

regions of the lymphoidal arterial sheaths. These regions of increased proliferative activity are supposedly at points where there has been formed a dense capillary network which is absent in other places in these sheaths (Thiel and Downey, '21). By the study of serial sections of spleens, in some cases of which the arteries had been injected with a carmine gelatin mass and others which had become highly congested with blood, it was demonstrable that the splenic nodule (Malpighian follicles) were always very well supplied with capillaries, which branches from the central artery, so that the first development of these nodules is undoubtedly due to an increase in nutrition and metabolic rate at these particular points.

The lighter colored central areas, which according to Thiel and Downey may also be peripherally located in the nodules, are not present in the nodules at first formed. In fact, the so-called 'germinal centers' are infrequently found at any time in splenic nodules as compared to nodules of the various tonsils and of the lymphatic nodes.

In splenic nodules, in which the lighter colored central areas are apparent, the accompanying artery is always seen to be extremely excentric to the nodular mass, and never in the lighter colored portion. Nodules which possess no lighter colored 'germinal center' are relatively smaller and the artery is usually located centrally in the nodule or nearly so. Neither is the so-called 'germinal center' a region of extraordinarily rich vascularity, as was found in the nodules at the time of their formation. In most cases no capillaries or blood could be demonstrated in these regions, the increase in size of the nodules and their increase in density at the periphery apparently inhibiting the vascular supply to the central portions. In some nodules the vascular supply was found to be very extensive throughout the entire nodule, as at the time of their formation, but these nodules never exhibited a 'germinal center.'

The histological appearance of these 'germinal centers' was first discussed by Flemming ('85), who found their lighter appearance to be due to the preponderance here of cells with large, vesicular nuclei, which were also relatively rich in cytoplasm

(lymphoid hemoblasts), so that the nuclei were farther separated from one another here than at the nodular periphery. This phenomenon was observed by later authors, and the large cells apparently peculiar to the areas were called by some the 'germinal center' cells (Czermack, '93; Hartmann, '14).

Thiel and Downey, in their study of the development of the mammalian spleen, found that the 'germinal centers' were areas in which the reticular tissue, particularly the reticular cells, were hypertrophied and were specially active in the reproduction and transformation into lymphocytes, thus disagreeing with Saxer ('96), who, in the study of the development of lymphatic nodes, said, "'The presence of a fixed mother tissue for the production of lymphocytes is neither practically demonstrable nor a theoretical postulate."

In the present study of the structure of the so-called 'germinal centers' as they appear in lymphatic nodules of the human spleen, it was found that, in these locations, the lymphocytes were much less closely packed together than at the periphery of the nodules. The cells in these centers were surely not predominately of the lymphoid hemoblast (lymphoblast, germinal center cell) type, as found by Flemming, Czermack, Hartmann, et al., the proportion between the small lymphocytes and the lymphoid hemoblasts remaining the same as at the periphery. Fibers of the reticular tissue are possibly more prominent in the 'germinal centers' than elsewhere in the nodules; this fact being due, it is thought, rather to the lesser density of the free lymphocytes and an increase of space between them, than to an hypertrophied condition of the reticulum. Nuclei of reticular cells were seen in the loose central portions, but not in unusual numbers, certainly not to a great enough extent to warrant a statement that the reticular cells are here specially active as a mother tissue productive of lymphocytes. It would seem that such prominence as the reticular tissue and cells may have in the so-called 'germinal centers' can be accounted for solely by the lesser number and density of the free lymphocytes in these places.

Evidences of proliferation of cells as indicated by the presence of mitotic figures are not as numerous, apparently, in splenic

lymphatic tissue as in other forms of lymphatic tissue. The regions where mitotic figures are most numerous are, therefore, not points of such special interest and significance as in other cases. Whenever mitotic figures were found, however, they were never located in the central areas, but in the denser peripheral portions of the nodules. There seemed to be no special points, at which the production of lymphocytes by mitosis was very active, and absolutely no evidence of such activity in the lighter central portions, so that the statement of Maximow ('09) and others, "that it is now a commonly accepted fact that small lymphocytes are formed by division of large lymphocytes in the germ centers," certainly cannot be applied to these areas in the lymphatic nodules of the spleen.

In examination of the lighter colored central areas in lymphatic tissue in other locations it has been found that the different concentration or character of the cells is not alone responsible for their appearance. Flemming noted large free cells in these places, similar to large lymphocytes, containing various deeply staining bodies in their cytoplasm, the 'tingible Körper.' Downey and Weidenreich ('12) also found here large cells, derived from reticular cells, which possessed phagocytic powers. Hartmann ('14) saw in the centers of intestinal lymphatic nodules large acidophilic cells with inclusions, which he thought were of a lipoid nature. These cells I also observed ('21) in similiar locations, and termed them acidophilic macrophages. They were considered as further differentiated, degenerating lymphoid hemoblasts, which had acquired phagocytic powers. The inclusions in the cytoplasm were thought to be remnants of nuclei of degenerating small lymphocytes.

Very few cells of such description were found in the nodules of splenic lymphatic tissue. A few large acid-staining cells which resembled the acidophilic macrophages of intestinal tonsils were found. Their nuclei were very similar to those found in the lymphoid hemoblasts, but did not give a typical staining reaction, staining rather with the acid stain. The basic cytoplasm staining reaction of the lymphoid hemoblasts was also changed to an acid reaction. Some intermediate stages could be found. No evidences

of their origin from reticular cells were found, although it might be considered as possible. In a very few instances deeply staining bodies were found in these cells. In some cases evidences of degeneration of small lymphocytes, such as their pycnotic nuclei and partial fragmentation, were found in these centers, and so, as before concluded in the case of inclusions of the macrophages of the intestinal tonsils, the inclusions of these macrophages were thought to be nuclear remnants of degenerating small lymphocytes.

The same significance is given to the large acid-staining macrophages in the so-called 'germinal centers' of the splenic lymphatic nodules, as to those similarly located in intestinal tonsillar tissue. The change in nuclear and cytoplasmic staining reaction from basic to acid, together with the acquisition of phagocytic powers is considered evidence of the further differentiation and degeneration of the lymphoid hemoblasts of these regions.

From these indications it would seem that the so-called 'germinal center' is not a region of special cell proliferation or transformation, but rather a center of degeneration. The fact that the lighter colored centers are present only in quite large nodules, which is associated with a decrease in the blood supply to these centers, the absence of mitotic figures, or a special mother tissue transforming into lymphocytes, together with the evidences of degeneration of small lymphocytes and of lymphoid hemoblasts, all point to an interpretation of these areas as degenerative rather than proliferative centers.

This interpretation certainly seems to be borne out in a study of pathological conditions. As is well recognized, pathological nodular growths, such as tubercular or sarcomatous nodules, when they become very large, have areas of caseous degeneration in the center. Extreme hypertrophy of lymphatic nodules as occurring in various blood diseases is coincident with degenerative changes in their central areas. In the history of infectious diseases involving the palatine or intestinal tonsils, it has been observed that the central portions of the nodules are the first areas to become affected, indicating that these points are areas of

lowered metabolic rate and resistance. It would seem somewhat illogical to consider these centers as areas of unusual proliferation due to excellent nutritive conditions and high metabolic rate and, at the same time, to admit them as possible foci of infection, associated with points of lowered resistance.

The lighter colored central areas, the so-called 'germinal centers,' when found in nodules of the lymphatic tissue of the spleen, are to be considered, then, as areas, in which the size of the nodules has caused a deficiency in the nutritive supply, as shown by the poor vascularity, which has lowered the metabolic rate to such an extent that evidences of degeneration are found, as shown by the presence of degenerative stages of small lymphocytes and lymphoid hemoblasts, and by the lesser density of lymphocytes there. These centers would consequently be points of lowered resistance and become possible foci of infection in the case of bacterial diseases invading the spleen.

The term 'germinal center,' therefore, seems non-descriptive of existing conditions, as it implies that it is a center of proliferation of lymphocytes, or transformation of other lymphoblastic tissue into lymphocytes, rather than a center of degeneration, due to poor nutritive conditions, as might be concluded from a study of these structures as found in the human spleen.

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PLATE 1

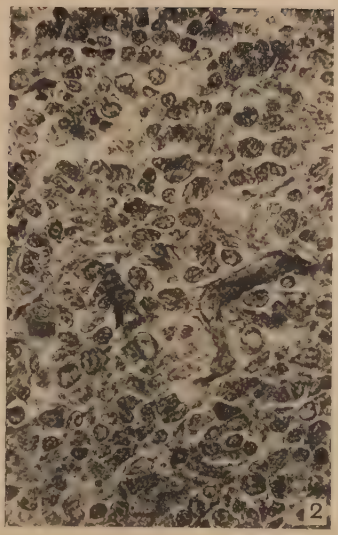
EXPLANATION OF FIGURES

1 Spleen, Homo, adult, showing a small lymphatic nodule, possessing no lighter colored center, with its accompanying artery located near the center of the nodule.

2 Spleen, Homo, adult, showing the character and density of cells in the lighter colored center of a lymphatic nodule. The reticular tissue is prominent here because of the lesser density of the free cells. Degenerative stages of lymphoid hemoblasts and of small lymphocytes (pycnotic nuclei) may be seen.

3 Spleen, Homo, adult, showing the comparative density of cells in the lighter colored center and in the peripheral portion of a lymphatic nodule. Several cells in various stages of degeneration of lymphoid hemoblasts (lightly staining nuclei) and of small lymphocytes (pycnotic nuclei) are found.

1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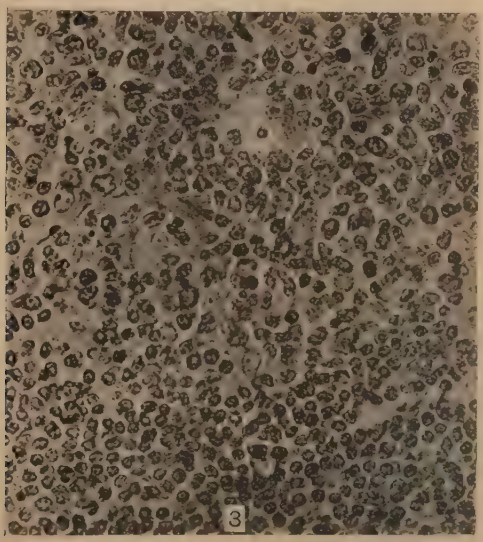
3

Resumen por el autor, H. S. Colton.

La anatomía de una rana con cinco patas

El presente trabajo es una descripción de un miembro que surjía en una cintura escapular accesoria situada en el extremo esternal del coracoides y precoracoides derecho. El miembro en cuestión, compuesto de húmero, radio-ulna y una mano reduplicada con ocho dedos, carecía de pigmento y músculos, excepto sobre la cabeza humeral, y aparentemente carecía de sensibilidad. El músculo pectoral del lado derecho presenta su orígen en el húmero accesorio y la cintura escapular accesoria, insertándose sobre estas estructuras y no sobre el esternón, como ocurre en el lado izquierdo, que es normal. A causa de la curiosa musculatura, el autor llega a la conclusión de que la anormalidad fué causada por una lesión del esternón.

Translation by José F. Nonidez
Cornell Medical College, New York.

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
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THE ANATOMY OF A FIVE LEGGED FROG

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FOUR FIGURES

In December 1921 I purchased from a collector of frogs a specimen of Rana pipiens with an extra fore limb protruding from the region of the right coracoid. Since it is by studying the abnormal structures which sometimes appear in animals that we are able to shed light on normal conditions, I thought it worth while to study the relation of this limb to the body of the animal.

While polymely is fairly common in the hind limb of the frog[1] it is much rarer in the fore limb. Why this difference exists is perfectly clear. The limb buds of the fore limb, growing as they do in the protected opercular chamber, are not subject to accident as are the buds of the hind limbs, exposed at all times to external conditions. Nevertheless, as can be seen from the table (page 248), polymely of the fore limb is not uncommon.

The frog in question, a small male (five cm. long) was kept alive in an aquarium. During the winter he fed upon spiders, meal worms and beetles. On April 20th he died from some unknown cause, perhaps by the water becoming too hot as the aquarium stood in a sunny place. While he was alive I had abundant opportunity to observe the actions and reactions of the supernumerary limb.

This limb armed with eight digits he kept folded across his chest (fig. 1). Except for the region on the proximal end of the limb no sign of pigment nor any musculature was visible. Indeed, pinching the limb with forceps showed that it was without sensation—no reaction could be observed. Indeed, so numb was

[1] Bateson, W. 1894 Materials for the study of variation. Lond. Macmillan.

TABLE 1

Cases of polymely of the fore limbs of Anura

AUTHOR	DATE	SPECIES	SYMMETRY	REGION	NUMBER OF DIGITS	SHOULDER-GIRDLE SUPERNUMERARY
Superville[1]	1740	?	Right	Scapular	?	?
Gervais[1]	1864	Pelobates cultripes	Left	Scapular, posterior	3	Coracoid and scapula
Lunel[1]	1868	Rana viridis	Left (pair)	Scapular (?)	4+4	scapula
Royal College of Surgeons[1]	1872[2]	?	?	Sternal	?	?
Mazza[1]	1888	Rana esamelica	Left (pair)		?	Scapula and coracoids
Bland Sutton[1]	1889	Rana temporaria	Left	Scapular	3	Coracoids
Bergendal[1]	1889	Rana temporaria	Left	Scapular	2	None
Tornier	1898	Rana esculenta	Right (pair)	Scapular, one ventral,	4	Coracoids
				one anterior	4	None
Washburn[1]	1899	Buffo columbiensis	Left	Scapular anterior	7	?
Johnson[1]	1901	Rana palmipes	Left and Right	Scapular "	4 ? destroyed	?
"		Rana halicinum	Right (pair)	Scapular (ventral)	?	?
Ergerman and Cox	1901	Rana pipiens	Right, ventral	Scapular	5	?
O'Donoghue	1910	Hyla aurea	Left, dorsal	Scapular	Destroyed	Scapula
"		Rana temporaria	Right, dorsal	Scapular	1	None
Wagner	1913	Rana pipiens	2 right 1 left	Sternal	4+4+4	?

[1] O'Donoghue, Chas. H. 1910 Instances of polymely in two frogs. Zool. Anz., vol. 35, pp. 759.

the limb that small pond snails attacked it. At times, they covered the limb, apparently feeding upon the epidermis and causing abrasions. The striking characteristics of the supernumerary limb were its position on the ventral side, the lack of musculature, the lack of pigment, and the lack of sensation.

Fig. 1 Photograph of frog, ventral surface. In the dark area anterior to the extra limb there is no muscle layer between the skin and the peritoneum; one looks directly into the body cavity.

The attack of the snails indirectly led to the frog's death. To protect him from the snails I removed him to an aquarium standing before a south window, and without land upon which to crawl. The next morning I found him dead. After his death I preserved him and dissected the arm. The shoulder

girdle proved quite normal on the left side and quite abnormal on the right. The right fore limb was normal, but the coracoid and precoracoid, instead of reaching to the sternum, were very short and supported a mass of cartilage with three centers of ossification. Of the three parts two, in their relationships, suggested coracoid and precoracoid, while the third, a forked structure which slid back and forth on a process from the sternum (fig. 2), could hardly be homologized with the scapula. Attempts to homologize these three parts with the normal shoulder girdle have proved unsatisfactory. With a glenoid cavity in the supernumerary shoulder girdle the extra humerus is articulated. On the distal end of this humerus is an indication of a joint, but no movement is possible. At this point the radio-ulnar is stiffly joined to the humerus. At the distal end of the radio-ulnar a mass of cartilage represents the carpals. From this mass eight digits extend, forming a reduplicated hand. The first digit is present as one, the metacarpal of the second is split on the end, the other three digits are all paired. Figure 3 shows the palmar surface of the double hand. Symmetrical reduplications of a similar sort have been reported by Harrison ('21) in his transplanted limb buds in Salamanders.

While the osteology is interesting the musculature throws more light on the origin of the limb. As I mentioned earlier, the only muscles present in the limb were found at its proximal end, the hand, the forearm and three quarters of the upper arm showed no trace of musculature that I could observe. Four muscles were concerned with the accessory shoulder girdle and the accessory humerus. Three of these muscles (fig 4 *A*, *B*, and *C*) were inserted in the crista deltoidea of the normal right humerus in the position of the normal three portions of the pectoralis muscle, which was absent. The anterior of these three muscles (*A*) had its origin on the accessory shoulder girdle, on the sternum, and on the supernumerary humerus. The second muscle (*B*) had its origin on the accessory shoulder girdle, while the third or posterior muscle (*C*) had its origin on the humerus of the supernumerary limb. A fourth muscle (*D*), in position a continuation of the second (*B*), had its origin on the accessory shoulder girdle

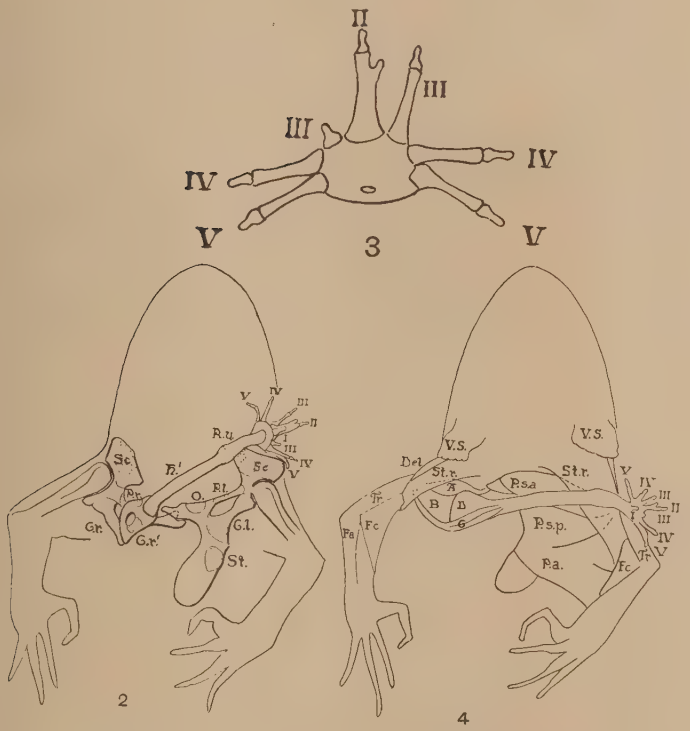

Fig. 2 Osteology of supernumerary limb, twice natural size.

C.l. Left coracoid
C.r. Right coracoid
C.r'. Supernumerary coracoid
H. Humerus
H'. Supernumerary humerus
O. Omosternum
P.l. Left precoracoid
P.r. Right precoracoid
R.u. Radio-ulnar
Sc. Scapula
St. Sternum

Fig. 3 Palmar surface of hand of supernumerary limb. This shows a twinning on an axis through the first and second digits. The first digit is hidden by the second. Four times natural size.

Fig. 4 The musculature of the fore limbs of the five legged frog. Twice natural size. *A*—Anterior muscle of the accessory limb. *B*—Median muscle of accessory limb. *C*—Posterior muscle of accessory limb. *D*—Muscle connecting supernumerary limb and shoulder girdle. *Del.*—Deltoid. *F.a.*—Flexor antibranchii. *F.c.*—Flexor carpi. *P.a.*—Pectoralis portio abdomalis. *P.s.a.*—Pectoralis portio sternalis anterior. *P.s.p.*—Pectoralis portio sternalis posterior. *St.r.*—Sterno-radialis. *Tr.*—Triceps. *V.S.*—Vocal Sack.

and passing over the head of the supernumerary humerus inserted on that bone. A comparison between the right or abnormal half of the frog with the left or normal half shows that the supernumerary limb is served by muscles homologous to the three portions of the normal pectoralis muscle,—portio sternalis anterior (fig. 4, *P.s.a*), portio sternalis posterior (*P.s.p.*), and portio abdominalis (*P.a.*). Ecker ('89). Supporting this view I found that on the right side the normal pectoralis muscle was lacking,—a fact which in life gave the frog a very peculiar appearance. In front of the supernumerary limb a dark patch was visible; there being no layer of muscle between the skin and the peritoneum, one looked directly into the coelom.

A review of cases of fore limb duplication (table 1) shows that the most common type arises in the scapular region and seems to be caused by a split in the limb bud. In two cases, in which, however, the anatomy was not studied, the limb is reported on the ventral side in the region of the sternum. Supernumerary fore limbs then in the Anura may be found in two regions, in the scapular region and in the sternal region.

Speculations on the possible origin of sternal limbs may be based on three sets of evidence; on the osteology, on the musculature and on the nerve supply. The limb may have been caused by a split in the limb bud involving the coracoid and precoracoid or it may have been due to an injury to the sternum. The osteology of our limb favors both hypotheses (fig. 2). Since the pectoralis muscle is normally innervated through the brachial nerve, were it possible to trace out the nerve to the pectoralis muscle, it would also favor both hypotheses, therefore a choice between the hypotheses depends upon the musculature. Since the origin of the pectoralis muscle is normally on the sternum, it would seem that the accessory limb has in some way been derived from the sternum. How this may take place is left for experimental analysis.

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Resumen por el autor, S. Naccarati

Sobre la relación entre el peso de las glándulas de secrección interna y los pesos del cuerpo y cerebro.

En el presente trabajo se consigna el peso de las glándulas de secrección interna de cinco especies de reptiles de la fauna italiana, a saber: Testudo graeca, Emys europaea, Tropidonotus natrix, Zamenis viridi flavus y Lacerta viridis. Los pesos absolutos de las glándulas, incluyendo también los del hígado, páncreas, riñones y bazo, y sus valores relativos con respecto al peso total y al del cerebro, representan la media de un número de animales examinados.

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AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
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ON THE RELATION BETWEEN THE WEIGHT OF THE INTERNAL SECRETORY GLANDS AND THE BODY WEIGHT AND BRAIN WEIGHT

SANTE NACCARATI

New York

In a previous work (1), in giving the average weight of the thyreoid gland of Emys europaea, I pointed out that the individual variations are very great. Weibgen (2), Tenchini and Cavatorti (3), Dustin and Zunz (4), and other investigators, who studied the thyreoid in man, found also the same great variability.

In this brief report I am giving the absolute weight of the endocrine glands and their value in relation to the body and brain weight in some species of reptiles of the Italian fauna which I had the occasion to study, namely, Emys europaea, Testudo graeca (Chelonia), Lacerta viridis (Lacertilia), Tropidonotus natrix, Zamenis viridi flavus (Ophidia). These organs show the same variability that was found in thyreoid.

Included in this report are also glands of external secretion, which are believed to possess also an internal secretory function.

In my study ovary was omitted because of the great variability due to the physiological condition of the animals. Pituitary, pineal, and parathyreoids were not considered on account of the difficulty of estimating them in the small species of reptiles studied by me.

Liver, pancreas, and spleen were in functional activity when the animals were killed.

Weighing operations were conducted within three hours from death.

The relative weight of each organ is referred to the body and to the brain weight in percentage values.

The data I give on reptiles represent averages from a given number of animals. These and especially those given by Welcker and Brandt (5), which are based sometimes on one or two animals, are too few for conclusions of a general nature.

In order to give an idea of the variability I have reported in table 6 the weight of the thyreoid in thirty Emys europaeas. Here, and in the case of the other animals, whose tables I have omitted for the sake of brevity, a positive correlation was found between the weight of the animal and that of the thyreoid.

Those who wish detailed and precise data on weights of viscera in man are referred to the works of Reid (6), Peacock (7), Gluge (8), Boyd (9), Bischoff (10), Blosfeld (11), Dieberg (12), Thoma (13), Gocke (14), Oppenheimer (15), Juncker (16), Richet (17), Kress (18), Buchanan and Daly (19), Greenwood (20), Castor (21), Greenwood and Brown (22), Bean (23), Bean and Baker (24).

In the tables, when not otherwise stated, the first column indicates the absolute weights in grams, the second gives the weight of the organ per 100 grams of body weight, the third represents the weight of the organ corresponding to 100 grams of brain substance.

TABLE 1

Emys europaea (aver. 30 animals)

1. Body weight	250		
2. Brain weight	0.25	0.1	
3. Thyroid	0.025	0.01	10
4. Thymus	0.08	0.03	32
5. Sex glands (testes, 17 animals)	0.61	0.24	244
6. Adrenals	0.028	0.01	11
7. Spleen	0.16	0.06	64
8. Pancreas	0.28	0.11	112
9. Liver	7.3	2.9	2920
10. Kidneys	1.38	0.5	552

TABLE 2

Testudo graeca (aver. 30 animals)

1. Body weight	320		
2. Brain weight	0.3	0.09	
3. Thyroid	0.036	0.011	12
4. Thymus	0.1	0.03	33
5. Sex glands (testes, 20 animals)	0.74	0.23	247
6. Adrenals	0.031	0.01	10
7. Spleen	0.18	0.06	60
8. Pancreas	0.3	0.09	100
9. Liver	8.5	2.65	2833
10. Kidneys	1.55	0.5	517

TABLE 3

Lacerta viridis (aver. from 15 animals)

1. Body weight	50		
2. Brain weight	0.121	0.24	
3. Thyroid	0.008	0.016	7
4. Thymus	0.03	0.06	24
5. Sex glands (testes, 8 animals)	0.2	0.4	165
6. Adrenals	0.02	0.04	16.5
7. Spleen	0.08	0.16	66
8. Pancreas	0.2	0.4	165
9. Liver	2.5	5.	2064
10. Kidneys	0.58	1.15	480

TABLE 4

Tropidonotus natrix (aver. from 20 animals)

1. Body weight	70		
2. Brain weight	0.1	0.14	
3. Thyroid	0.01	0.014	10
4. Thymus	0.015	0.02	15
5. Sex glands (testes, 12 animals)	0.23	0.3	330
6. Adrenals	0.035	0.05	35
7. Spleen	0.12	0.17	120
8. Pancreas	0.25	0.35	250
9. Liver	2.8	4.	2800
10. Kidneys	0.88	1.4	980

TABLE 5

Zamenis viridiflavus (aver. 6 animals)

1. Body weight	220		
2. Brain weight	0.209	0.09	
3. Thyroid	0.045	0.02	21
4. Thymus	0.075	0.03	30
5. Sex glands (testes)	0.48	0.21	230
6. Adrenals	0.08	0.04	38
7. Spleen	0.125	0.06	60
8. Pancreas	0.415	0.18	200
9. Liver	4.825	2.2	2300
10. Kidneys	1.935	0.8	830

TABLE 6

Weight of thyreoid in Emys europaea

NO.	SEX	WEIGHT OF THE ANIMAL	ABSOLUTE WEIGHT OF THYREOID	RELATIVE WEIGHT OF THYREOID (PER 100 GRAMS OF BODY WEIGHT)
1	F	179	0.025	0.014
2	M	195	0.009	0.005
3	M	200	0.016	0.008
4	M	205	0.020	0.010
5	M	207	0.023	0.011
6	F	220	0.018	0.008
7	F	228	0.029	0.013
8	M	231	0.035	0.015
9	M	232	0.018	0.007
10	F	238	0.026	0.010
11	M	244	0.021	0.009
12	M	246	0.030	0.012
13	M	248	0.025	0.010
14	M	250	0.020	0.008
15	F	255	0.028	0.011
16	F	260	0.025	0.009
17	M	265	0.027	0.010
18	F	271	0.023	0.008
19	F	274	0.033	0.012
20	M	276	0.029	0.010
21	M	279	0.041	0.015
22	F	281	0.032	0.011
23	F	288	0.034	0.012
24	M	290	0.037	0.013
25	F	296	0.038	0.013
26	F	298	0.031	0.010
27	M	309	0.044	0.014
28	M	326	0.039	0.012
29	M	349	0.042	0.012
30	F	375	0.075	0.020

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